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CARBOHYDRATE THIOETHERS

1-DEOXY-1-THIOETHYL-D-FRUCTOSE[1]

BY SAMUEL B. BAKER

ABSTRACT

1-Deoxy-1-thioethyl-D-fructose was synthesized by two distinctly different methods. Less than the usual hindrance of the toluenesulphonyloxy group on the C-1 position in 2,3-4,5-diisopropylidene-1-*O*-*p*-toluenesulphonyl-D-fructose was found to occur in the presence of sodium ethanethiolate.

A previous (1) publication described the synthesis of a thioalkyl aldose, namely, 6-deoxy-6-thioethyl-D-galactose. Extension of this investigation into the ketose series resulted in the synthesis of 1-deoxy-1-thioethyl-D-fructose (III) by two distinctly different methods.

The first method employed 2,3-4,5-diisopropylidene-1-*O*-*p*-toluenesulphonyl-D-fructose (I). A solution of the latter substance in anhydrous N,N-dimethylformamide on treatment with freshly prepared sodium ethanethiolate yielded 2,3-4,5-diisopropylidene-1-deoxy-1-thioethyl-D-fructose (II), but only after heating at 100° for 30 hr. This latter reaction was exceedingly slow when compared with the reaction of sodium ethanethiolate and 1,2-3,4-diisopropylidene-6-*O*-*p*-toluenesulphonyl-D-galactose (I). The latter reaction was complete in two hours. The sluggishness of the ketose reaction is reminiscent of the nonreaction of the same fructose derivative with sodium iodide in acetone (4).

The second method of synthesis of III made use of so-called "glutose". This substance is essentially a mixture of fructose anhydrides (6, 7). Treatment of the amorphous "glutose" mixture with an excess of sodium ethanethiolate in anhydrous methanol yielded a small quantity of a substance that proved to be identical to that obtained from I.

EXPERIMENTAL

2,3-4,5-Diisopropylidene-D-fructose (3)

This substance was prepared according to the method of Glen, Myers, and Grant (3) with slight modification. A yield of 347 gm. (89%) was obtained from 270 gm. fructose. The melting point 96–97° and $[\alpha]_D^{22°}$ −32.6° (H_2O: *c*, 1.013) agreed with the constants reported in the literature (2) for 2,3-4,5-diisopropylidene-D-fructose.

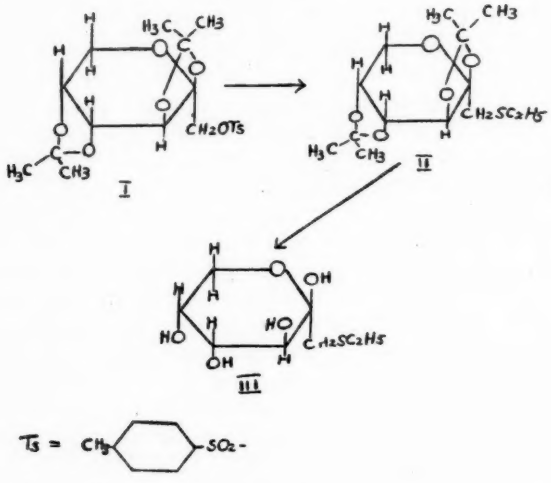
[1]*Manuscript received May 13, 1955.*
Contribution from the Division of Organic Chemistry, Research Institute, Montreal General Hospital, Montreal, Que.

*2,3-4,5-Diisopropylidene-1-O-p-toluenesulphonyl-*D*-fructose* (*I*) (5)

2,3-4,5-Diisopropylidene-D-fructose (179 gm.) was tosylated with *p*-toluenesulphonyl chloride (135 gm.) in anhydrous pyridine. The reaction mixture was treated in the usual manner and the crude air-dried product was recrystallized from methanol yielding 192 gm. (66%). A second recrystallization from aqueous ethanol gave m.p. 81–82° and $[\alpha]_D^{22°}$ −27.2° (ethanol: *c*, 2.693). The literature (5) reports m.p. 83° and $[\alpha]_D$ −27.1° (ethanol).

*2,3-4,5-Diisopropylidene-1-deoxy-1-thioethyl-*D*-fructose* (*II*)

2,3-4,5-Diisopropylidene-1-*O*-*p*-toluenesulphonyl-D-fructose (I) (97.5 gm.) and freshly prepared sodium ethanethiolate (35 gm.) were dissolved in hot N,N-dimethylformamide (275 cc.). The mixture was heated on the steam bath for 30 hr. and then added to cold water (3000 cc.). An oil separated and the mixture was extracted twice with ether. The ethereal solution was dried over



anhydrous sodium sulphate, filtered, and concentrated *in vacuo*. The residual sirup was then heated at 100° for six hours at 10 mm. pressure to remove any N,N-dimethylformamide. The main product was distilled at 96–100° and 0.04 mm. and the yield was 54.4 gm. (76%). A portion of the nearly colorless distillate was fractionally distilled through a 20 cm. Widmer column and practically all distilled at 107° and 0.07 mm. The constants were: $\eta_D^{26°}$ 1.4823 and $[\alpha]_D^{20°}$ −58.4° (chloroform: *c*, 9.4212). Anal.: Calc. for $C_{14}H_{24}SO_5$: C, 54.27; H, 7.89; S, 10.52. Found: C, 54.02; H, 7.93; S, 10.44.

*1-Deoxy-1-thioethyl-*D*-fructose* (*III*)

2,3-4,5-Diisopropylidene-1-deoxy-1-thioethyl-D-fructose (20 gm.) was added to 1 *N* sulphuric acid (1000 cc.). The mixture was stirred for 18 hr. at 50°, then cooled to room temperature and carefully neutralized with barium hydroxide – barium carbonate. The solution was not allowed to become alkaline

at any time. The barium sulphate and carbonate mixture was removed by filtration, the filter washed several times with hot water, and the combined filtrates and washings concentrated to a thick sirup *in vacuo* at 40°. The sirup, after it had been kept for three days in an evacuated desiccator over calcium chloride, crystallized. The hard mass was dissolved in a small volume of hot acetone and filtered. Slow cooling of the filtrate caused crystallization. The nearly pure product, having a melting point of 86–88°, was recrystallized once more from anhydrous acetone – ether mixture when the melting point was raised to 87.5–88.5° and it showed $[\alpha]_D^{22°}$ −71.3° → −62.8° (24 hr.) (water: *c*, 3.1548). Anal.: Calc. for $C_8H_{16}SO_5$: C, 42.85; H, 7.14; S, 14.29. Found: C, 42.66; H, 7.31; S, 14.3.

D-Glucose Phenylosazone from 1-Deoxy-1-thioethyl-D-fructose

1-Deoxy-1-thioethyl-D-fructose (2 gm.) was dissolved in water (20 cc.). Acetic acid (5 cc.) and redistilled phenylhydrazine (4 cc.) were added and the clear solution heated at 80° for 30 min. Ethanethiol was evolved and precipitation occurred. The reaction mixture was cooled to 20°, filtered, and the filter washed with cold water. The yield of air-dried material was 2.3 gm. (71%). A sample was recrystallized twice from hot dilute methanol and it melted at 205–207°. A mixed melting point determination with authentic D-glucose phenylosazone was 204–207°.

1-Deoxy-1-thioethyl-D-fructose from "Glutose" (6, 7)

Ethanethiol (40 cc.) was added to cold 0.7 *N* sodium methylate solution (500 cc.). "Glutose" (6, 7) (21 gm.) was added and the brown solution heated under reflux on the steam bath for two hours. The reaction mixture was cooled to 10°, neutralized carefully in the fume cabinet with 3 *N* hydrochloric acid, and then concentrated *in vacuo* at 30°. Final traces of water were removed by ethanol–benzene azeotropic distillation. The dry sirup was extracted with boiling anhydrous acetone and the filtrate was concentrated *in vacuo* to a thick sirup. The latter sirup was again extracted with hot acetone and the filtered solution was decolorized with activated charcoal. The yellow filtrate obtained was concentrated to approximately 50 cc., seeded with several crystals of 1-deoxy-1-thioethyl-D-fructose (III), and allowed to stand stoppered at room temperature for about three weeks. During this time crystallization occurred. The crystals were removed by filtration and the filter was washed with cold (0°) acetone. The crude product was twice recrystallized from acetone–ether and after drying it melted at 87–88.5°. The mixed melting point with 1-deoxy-1-thioethyl-D-fructose, obtained previously, was 86–88°. The yield was 0.36 gm.

Tetraacetyl-1-deoxy-1-thioethyl-D-fructose

1-Deoxy-1-thioethyl-D-fructose (5 gm.) was dissolved in anhydrous pyridine (25 cc.). The solution was cooled to −20° and acetic anhydride (25 cc.) was added. The reaction mixture was kept at −20° for one hour, allowed to stand at 22° for 22 hr., and finally added to cold water. The separated sirupy product did not crystallize and the aqueous mixture was extracted four times with ether (100 cc.). The combined ethereal solutions were washed with cold 2%

hydrochloric acid, water, and saturated potassium bicarbonate and then dried over anhydrous sodium sulphate. The ethereal solution, after filtration, was concentrated to dryness by slow evaporation in a desiccator and the sirupy mass solidified, in a yield of 5.9 gm. (68%). It was recrystallized twice from anhydrous ether and it melted at 133–134° and $[\alpha]_D^{22°}$ −47.6° (chloroform: *c*, 1.8492). Anal.: Calc. for $C_{16}H_{24}SO_9$: C, 48.98; H, 6.12; S, 8.19; CH_3CO, 43.9. Found: C, 48.60; H, 6.41; S, 8.2; CH_3CO, 43.74.

*Tetrabenzoyl-1-deoxy-1-thioethyl-*D*-fructose*

1-Deoxy-1-thioethyl-D-fructose (5 gm.) was dissolved in anhydrous pyridine (50 cc.). The mixture was cooled to −20° and benzoyl chloride (13 gm.) was added in one portion. The mixture was allowed to stand at −20° for two hours, then overnight at 22°. The reaction product was isolated as above and the resulting sirup was crystallized from methanol. The separated crystalline mass was recrystallized from hot methanol and the nearly pure product, 8.1 gm. (57%), after an additional recrystallization from a large volume of ether – petroleum ether (1:1) melted at 103–104° and $[\alpha]_D^{22°}$ +61.9° (chloroform: *c*, 2.2844). Anal.: Calc. for $C_{36}H_{32}SO_9$: C, 67.5; H, 5.0; S, 5.0; C_6H_5CO, 65.62. Found: C, 67.34; H, 5.1; S, 4.96; C_6H_5CO, 65.6.

ACKNOWLEDGMENT

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THE TERMINAL AMINO ACIDS OF WHEAT GLIADIN[1]

By L. K. Ramachandran[2] and W. B. McConnell

ABSTRACT

Wheat gliadin has been found by two different methods to contain three N-terminal histidine residues for each molecular weight of 27,000. Trace amounts of N-terminal aspartic acid, glutamic acid, alanine, valine, and serine were also detected in the preparation used. Hydrolysis in boiling hydrochloric acid partially destroyed the di-2,4-dinitrophenyl derivative of histidine. Losses of from 5% to 25% occurred depending upon the time and conditions of hydrolysis. Carboxypeptidase did not release free amino acids from wheat gliadin but qualitative evidence for the occurrence of C-terminal glutamic acid and C-terminal "leucine" was obtained.

It has been reported by Koroś (9) that wheat gliadin contains three free amino groups and that these belong to the three N-terminal histidine residues. Although doubt exists that gliadin is composed of a homogeneous and unique molecular species the observations of Koroś would point to the existence of at least one characteristic feature in gliadin. Because details of Koroś's work were unavailable independent experiments were done to determine the nature of the N-, and also of the C-, terminal amino acids of gliadin. Since the completion of the present work Koroś's results have been questioned (6) and it was reported that Sanger's method revealed one N-terminal tyrosine residue in gliadin from *Triticum durum*, and two N-terminal residues, one each of glutamic acid and tyrosine, in gliadin from *Triticum vulgare*; no N-terminal histidine was detected. Results recorded in the present communication support the earlier report (9) that histidine is the major N-terminal amino acid in gliadin. In addition, evidence presented indicates that glutamic acid and "leucine" occupy the C-terminal positions.

EXPERIMENTAL

Gliadin was prepared by the method of Blish and Sandstedt (4). The sample contained 17.64% nitrogen on a moisture and ash-free basis. The ninhydrin colorimetric procedure (8) and the Van Slyke nitrous acid method (11 min. reaction) indicated 3.18 and 3.32 free amino groups, respectively, for an assumed molecular weight of 27,000.

Identification of N-Terminal Groups Using 2,4-Dinitrofluorobenzene

DNP-gliadin was prepared using the reaction conditions described by Porter (14). Two grams of the protein and 2 gm. $NaHCO_3$ were suspended in 20 ml. water, 40 ml. of a 10% (w/v) solution of DNFB in ethanol were added, and the mixture was shaken for two hours at room temperature. The reaction mixture was exhaustively dialyzed against distilled water and the precipitated protein collected, washed, and dried (yield 2.0 gm.). It was calculated from

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[2]*National Research Council of Canada Postdoctorate Fellow, 1953–55.*

the amide nitrogen content (14) that 119.9 mgm. of DNP-gliadin corresponded to 100 mgm. of the original gliadin. Samples of DNP-gliadin (100 mgm.) were hydrolyzed in 10 ml. of boiling 5.7 *N* hydrochloric acid for four hours or for 24 hr. Another sample was hydrolyzed in 12 *N* acid in a sealed tube at 105° C. for 24 hr. (14). The DNP-amino acids in the hydrolyzates were fractionated into ether-soluble and acid-soluble materials and the fractions were chromatographed by each of three previously used methods (2, 3, 13).

DNP-histidine was the only amino acid derivative found in the acid-soluble fraction. Trace amounts of DNP-aspartic acid, glutamic acid, alanine, valine, and serine were detected in the ether-soluble material and, although gliadin contains at least two residues of lysine per molecule, no ε-DNP-lysine was found. The identity of the DNP-histidine was established by hydrolyzing it with concentrated ammonia (12) in a sealed tube and chromatographing the resulting amino acid on paper.

The yields of various 2,4-dinitrophenyl derivatives obtained from the treated gliadin are recorded in Table I. The calculations for the histidine are

TABLE I

YIELD OF DNP-DERIVATIVES FROM ACID HYDROLYZATES OF DNP-GLIADIN

Derivative	Yield: moles/27,000 gm.	
	4 hr. hydrolysis	24 hr. hydrolysis
Di-DNP-histidine (N-terminal histidine)	3.1	2.9
Other DNP-amino acids*	0.17	0.11
Dinitrophenol, dinitroaniline, etc.†	1.3	1.0

**Calculated as DNP-aspartic acid.*
†*Calculated as dinitrophenol.*

based on optical density measurements at 360 mμ and reference to the calibration curve for di-DNP-histidine. The DNP-amino acids found in traces are calculated as DNP-aspartic acid. Values for histidine are corrected for hydrolytic losses by increasing them by 25% (see below). It was also found that only 71% of the expected histidine derivative could be estimated in a dinitrophenylation of histidine done under the same conditions as employed with gliadin. To compensate for this low recovery in dinitrophenylation the yields of di-DNP-histidine were also multiplied by the factor 1.41. Because of the large corrections the results in Table I are only approximate. Chromatography on a silicic acid column was used to separate trace amounts of DNP-amino acids from the relatively large amounts of dintrophenol and dinitroaniline found in the ether-soluble fraction (11). No corrections have been applied for hydrolytic losses of the latter materials.

Effect of Acid Hydrolysis on Di-DNP-histidine

Di-DNP-histidine, with the reported physical constants (15), was prepared according to the method of Porter. Twenty-five milligrams was hydrolyzed with 20 ml. of 5.7 *N* HCl. The hydrolyzates were extracted with ether to remove artifacts like dinitroaniline and dinitrophenol, and the di-DNP-histidine

remaining in the acid phase was determined by spectrophotometry. For time intervals of 3.5, 6.5, and 24.0 hr. the destruction observed was 0.0, 5.0, and 12.0% respectively. When the same weight of the pure derivative was hydrolyzed in the presence of 40 mgm. gliadin the destruction observed was 25.0% for 24 hr. The latter figure has been used to correct for hydrolytic losses.

Application of the Edman Method (7) to N-Terminal Residue Identification

One gram gliadin was dissolved in 4 ml. 50% pyridine, the pH was adjusted to 8.6 with 0.1 *N* alkali, and 0.2 ml. of phenylisothiocyanate was added with vigorous stirring. The temperature was maintained at 40° C., and alkali was added as required to keep the pH at 8.6. When consumption of alkali ceased, the reaction mixture was extracted twice with benzene and the protein precipitated with acetone. The dry phenylthiocarbamyl protein was then suspended in 9 ml. of anyhydrous nitromethane containing 4% HCl by weight. After an hour of agitation, the material was filtered and washed with a few milliliters of the solvent. The combined filtrates were evaporated *in vacuo* to remove all traces of solvents and HCl. The residue was dissolved in EtOH. Absorption at 270 mμ indicated a content of 3.2 M. of phenylthiohydantoin per 27,000 gm. of the protein. Paper chromatography of the material (10) showed that PTH-histidine was the major component. Its identity was confirmed by barium hydroxide hydrolysis to the free amino acid. Stepwise degradation of the residual protein was carried out but during the second and third treatments an abnormally high yield of phenylthiohydantoin (of the order of five moles) was obtained. No detailed identification of the material was attempted, but paper chromatography of barium hydroxide hydrolyzates indicated the presence of several ninhydrin positive spots.

C-Terminal Residues of Gliadin

Action of carboxypeptidase on gliadin.—One-half gram of gliadin was suspended in 20 ml. water, the pH was adjusted to 7.7 with 0.2 *N* NaOH, and the mixture was incubated with 0.2 ml. of carboxypeptidase suspension (12.6 mgm./ml. Armour). In another experiment the same weight of protein was suspended in 20 ml. of 1% (w/v) sodium bicarbonate. No liberation of amino *N* could be detected by the Van Slyke method during a seven-hour period, and no free amino acids could be detected in the reaction mixture by paper chromatography. It was therefore concluded that the C-terminal amino acid residues in gliadin, if any, were not susceptible to hydrolysis by carboxypeptidase under the conditions used.

Application of the method of Schlack and Kumpf (1) to gliadin.—One gram of the protein was mixed with 0.3 gm. of pulverized anhydrous ammonium thiocyanate in 77 ml. of an AcOH: Ac_2O (9: 1) mixture, and the reaction mixture was stirred at 45° C. for four hours. At the end of this period 30 ml. of HCl was added dropwise with stirring and the material heated on a steam bath for one hour. The product was dried *in vacuo*, the residue dissolved in 60 ml. of 0.25 *M* phosphate (pH 6.5) and extracted five times with equal volumes of ethyl acetate. The combined extracts were washed once with an equal volume of water and dried, and the residue was hydrolyzed with 2.5 ml.

of 1.25 *N* $Ba(OH)_2$ at 140° C. in a sealed tube. The hydrolyzate was neutralized with carbon dioxide and heated for 10 min. on a steam bath to destroy carbamic acids. Aliquots of the suspension were examined by paper chromatography (BuOH–AcOH–H_2O, 4:1:5). The only amino acids detected were glutamic acid and "leucine". The amounts of these amino acids were estimated by ninhydrin colorimetry using a standard curve for the two amino acids obtained under the same conditions. One-half mole of glutamic acid and 0.45 mole of "leucine" were found for each 27,000 gm. gliadin. No corrections were applied.

Application of the lithium borohydride reduction method of Chibnall (5) indicated the presence of glutamic acid and "leucine" in C-terminal positions. Appreciable traces of other amino acids were also detected. Using a nine-fold excess of diazomethane for esterification of the protein and a 13-fold excess of $LiBH_4$ for the reduction in tetrahydrofuran, the total yield of α-amino alcohols was 0.654 mole per 27,000 gm. protein.

DISCUSSION

The present work supports the observations of Koroś (9) that gliadin of molecular weight 27,000 contains three free amino groups and that these belong to histidine. From this result, it was expected that three C-terminal amino acids could be detected. It was surprising, therefore, that carboxypeptidase did not liberate any free amino acids from the protein; particularly in view of the finding that leucine and glutamic acid were detected as C-terminal residues by the chemical method (1). Recoveries by the latter method are known to be very poor, and since only about 0.5 mole of C-terminal leucine and glutamic acid was found the data were not adequate for evaluation of the amounts of C-terminal leucine and C-terminal glutamic acid in gliadin. It is to be noted that Baptist and Bull (1) could not identify glutamic acid in glutathione or recover glutamic acid from glutamine by the Schlack and Kumpf method. The present identification of the C-terminal acids would therefore be tentative.

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THE PREPARATION OF GUANIDINE FROM UREA, AMMONIUM CHLORIDE, ALUMINUM SULPHATE, AND AMMONIA UNDER PRESSURE[1]

By Jean L. Boivin

ABSTRACT

Guanidine has been synthesized by heating under ammonia pressure an intimate mixture of finely powdered urea, ammonium chloride, and aluminum sulphate. Good yields of guanidine as the hydrochloride salt have been obtained over a wide range of conditions. The mechanism of this synthesis is briefly discussed.

INTRODUCTION

If urea is heated under ammonia pressure, it is much more stable at high temperature because of the stabilizing effect of ammonia (1). The preparation of guanidine about to be described makes use of aluminum sulphate to assist the removal of water from the reaction. This starting material can be produced cheaply from bauxite, clay, and similar materials containing aluminum.

DESCRIPTION OF METHOD

By heating urea, ammonium chloride, and aluminum sulphate together under ammonia pressure at 300° C. for some time, yields of guanidine as high as 70% have been obtained. The reaction product was boiled with ammoniacal water in order to precipitate aluminum hydroxide (Diagram 1). The clear filtrate, which contained guanidine hydrochloride, ammonium sulphate, and unreacted urea and ammonium chloride, was evaporated to dryness. The residue was treated with liquid ammonia and the mixture filtered free from ammonium sulphate. By evaporation of the liquid ammonia solution, a residue was left containing guanidine hydrochloride together with unreacted urea and ammonium chloride. This crude guanidine hydrochloride was transformed into guanidine nitrate by treating its saturated aqueous solution with a quantity of ammonium nitrate equivalent to its guanidine content. After guanidine nitrate was removed the filtrate was evaporated to dryness and the residue heated to 250° C. This material was nearly pure ammonium chloride, suitable for use in a subsequent cycle.

The aluminum hydroxide and ammonium sulphate, individually isolated from the reaction products as indicated above, were heated together at 350° C. to regenerate aluminum sulphate (5) according to the following equation:

$$2Al(OH)_3+3(NH_4)_2SO_4 \rightarrow Al_2(SO_4)_3+6H_2O+6NH_3. \qquad [1]$$

The ammonia evolved during heating can be dried and returned to the reactor.

EFFECT OF TEMPERATURE

In order to explore the effect of the temperature of heating, mixtures consisting of equimolar quantities of urea and ammonium chloride and various

[1]*Manuscript received June 2, 1955.*
Contribution from the Organic Section of Canadian Armament Research and Development Establishment, Valcartier, Quebec. Issued as C.A.R.D.E. Report No. 50/51, 1951.

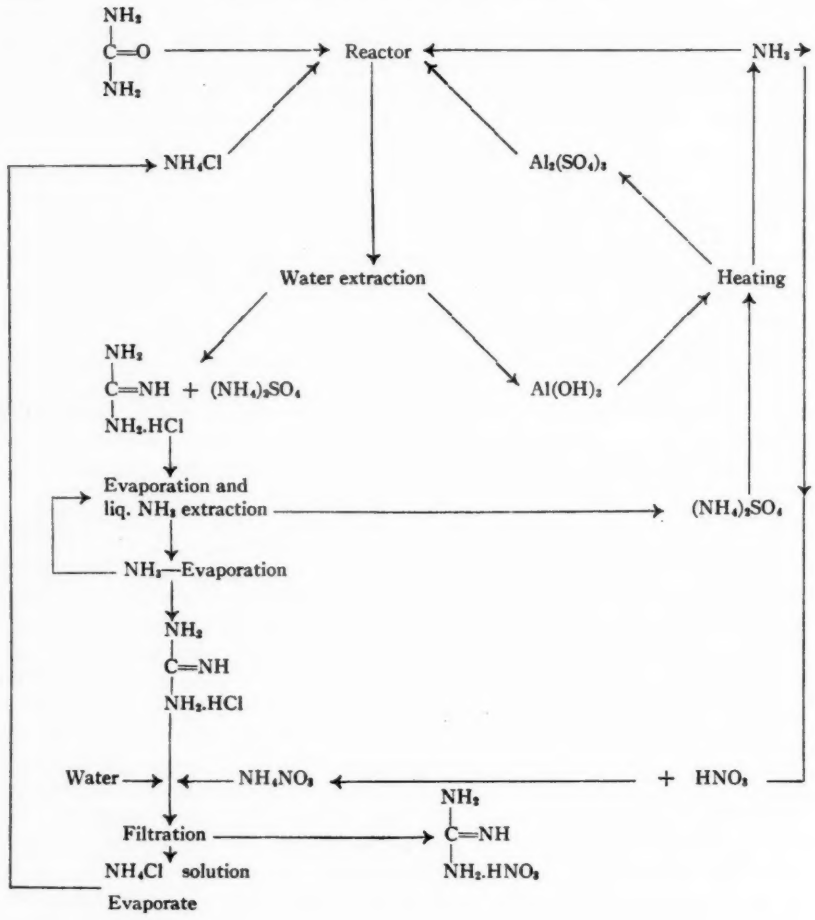


Diagram 1

amounts of aluminum sulphate were heated together under ammonia pressure. Table I shows the compositions of the mixtures and the conditions of the reaction. At high temperature, melamine is produced at the expense of guanidine. The yields of guanidine increase with temperature up to a maximum value, beyond which the formation of melamine becomes increasingly significant. The low yields shown here are ascribed to the fact that the ingredients were coarse and not thoroughly mixed. However, the table shows that increasing yields of guanidine were obtained with increasing amounts of aluminum sulphate.

TABLE I

EFFECT OF TEMPERATURE

Urea: 0.10 mole; NH_4Cl: 0.10 mole; time: 30 min.; pressure: 1000 p.s.i.g.

$Al_2(SO_4)_3$, mole	Temp., ° C.	Yield from urea, %	
		Guanidine	Melamine
0.0166	225	4	Nil
	250	10	Nil
	275	17	Nil
	300	36	10
	325	41	28
	350	29	35
0.033	250	14	Nil
	275	32	Nil
	300	45	Nil
	325	48	16
	350	38	20
0.05	225	Traces	Nil
	250	26	Nil
	275	40	Nil
	300	55	Nil
	325	63	37
	350	45	42

EFFECT OF CONCENTRATION OF UREA

By varying the molar quantities of urea added to a constant mixture of ammonium chloride and aluminum sulphate, as can be seen in Table II, the optimum yield was attained from equimolar amounts of urea and ammonium chloride. When urea was added in excess of this value, melamine was formed together with cyanuric acid.

TABLE II

EFFECT OF CONCENTRATION OF UREA

NH_4Cl: 0.10 mole; $Al_2(SO_4)_3$: 0.05 mole; temp.: 300° C.; time: 30 min.; pressure: 1000 p.s.i.g.

Urea, mole	Yield from urea, %	
	Guanidine	Melamine
0.05	43	Nil
0.10	55	Nil
0.15	34	23
0.20	31	22
0.30	22	20

EFFECT OF CONCENTRATION OF AMMONIUM CHLORIDE

It has been found (Table III) that increasing quantities of ammonium chloride give increasing yields of guanidine, melamine being absent under these conditions. There is a great increase in yield as ammonium chloride increases from 0.05 mole to the stoichiometric amount of 0.10 mole. The better yields obtained here are attributed to thorough mixing of solid ingredients before reaction took place.

TABLE III

EFFECT OF AMMONIUM CHLORIDE

Urea: 0.10 mole; $Al_2(SO_4)_3$: 0.033 mole; temp.: 300° C.; time: 30 min.; pressure: 1000 p.s.i.g.

NH_4Cl, mole	Guanidine yield from urea, %
0.05	39
0.10	68
0.15	70
0.20	75
0.25	68
0.30	65

EFFECT OF TIME OF HEATING

This reaction is dependent on the period of heating (Table IV). It is more complete when the reaction time is longer. The yields increase with time and also with temperature.

TABLE IV

Urea: 0.10 mole; NH_4Cl: 0.10 mole; $Al_2(SO_4)_3$: 0.033 mole; pressure: 1000 p.s.i.g.

Temp., ° C.	Time, min.	Guanidine yield from urea, %
275	30	58
	60	62
	90	64
	120	68
300	30	68
	45	70
	60	72
	90	79

EFFECT OF PRESSURE

The pressure factor is quite important in this reaction. There is evidence (Table V) that the yields are better at higher pressure. At atmospheric pressure, neither guanidine nor melamine was produced. At high pressure, yields were improved very significantly.

TABLE V

EFFECT OF PRESSURE

Urea: 0.10 mole; NH_4Cl: 0.10 mole; $Al_2(SO_4)_3$: 0.033 mole; temp.: 300° C.; time: 30 min.

Pressure, p.s.i.g.	Guanidine yield from urea, %
0	0
100	33
200	37
400	57
1000	68
2000	72

MECHANISM OF THE REACTION

Although the mechanism of this reaction, involving four starting materials, is not fully understood, some suggestions may be offered as to the possible

intermediate compounds. In the absence of ammonium chloride, the heating of urea with aluminum sulphate and ammonia failed to give guanidine under the same conditions. Likewise, urea, ammonium chloride, and ammonia gave low yields of guanidine under these conditions of temperature and time; even with the long period of heating employed by Blair (1), these reactants gave only 23% guanidine. When both ammonium chloride and aluminum sulphate are present, however, yields as high as 75% can be obtained in a period of only an hour or so.

It is believed that in the presence of ammonia vapor, as in the present reaction, ammonium chloride and aluminum sulphate react as in the following reaction:

$$6NH_4Cl + Al_2(SO_4)_3 \longrightarrow 2AlCl_3 + 2(NH_4)_2SO_4. \qquad [2]$$

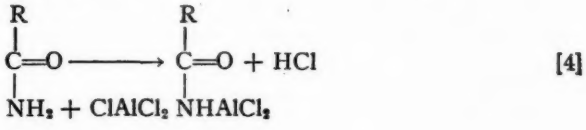
If then urea is present (6) and a small conversion to guanidine takes place, liberating water, the latter can react with aluminum chloride:

$$2AlCl_3 + 3H_2O \longrightarrow Al_2O_3 + 6HCl. \qquad [3]$$

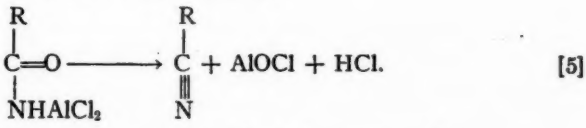
Accordingly, reaction is displaced towards the right, i.e. formation of guanidine is facilitated.

By analogy with the sulphamate synthesis of guanidine from urea (3) and also with the preparation of nitriles from amides by the action of sulphamates (2), it might be expected that an intermediate compound, comprising a substituted urea, could be involved.

Norris and Klemka (4) have shown that nitriles are obtained from amides and aluminum chloride through the formation of an intermediate compound, according to the following equation:

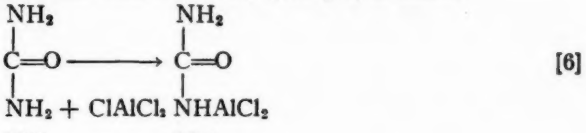


$$\begin{array}{l} R \\ | \\ C{=}O \\ | \\ NH_2 \end{array} + ClAlCl_2 \longrightarrow \begin{array}{l} R \\ | \\ C{=}O \\ | \\ NHAlCl_2 \end{array} + HCl \qquad [4]$$

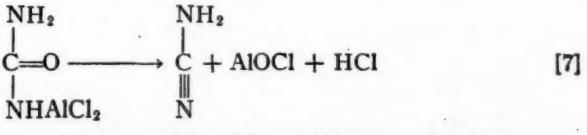


$$\begin{array}{l} R \\ | \\ C{=}O \\ | \\ NHAlCl_2 \end{array} \longrightarrow \begin{array}{l} R \\ | \\ C \\ \| \\ N \end{array} + AlOCl + HCl. \qquad [5]$$

Urea, which is an amide, could react similarly giving cyanamide:



$$\begin{array}{l} NH_2 \\ | \\ C{=}O \\ | \\ NH_2 \end{array} + ClAlCl_2 \longrightarrow \begin{array}{l} NH_2 \\ | \\ C{=}O \\ | \\ NHAlCl_2 \end{array} \qquad [6]$$



$$\begin{array}{l} NH_2 \\ | \\ C{=}O \\ | \\ NHAlCl_2 \end{array} \longrightarrow \begin{array}{l} NH_2 \\ | \\ C \\ \| \\ N \end{array} + AlOCl + HCl \qquad [7]$$

which under the actual condition should yield guanidine or melamine.

The above suggestions, though tentative, seem to offer an explanation of the observation that whereas neither ammonium chloride nor aluminum sulphate alone is effective, the two together produce a high yield of guanidine from urea and ammonia in a relatively short time of reaction.

It is believed that guanidine hydrochloride is formed from urea according to the following over-all equation:

$$3\,\underset{\displaystyle NH_2}{\overset{\displaystyle NH_2}{|}}\!\!>\!C\!=\!O + 3NH_4Cl + Al_2(SO_4)_3 + 6NH_3 \rightarrow 3\,\underset{\displaystyle NH_2.HCl}{\overset{\displaystyle NH_2}{|}}\!\!>\!C\!=\!NH + Al_2O_3 + 3(NH_4)_2SO_4. \qquad [8]$$

EXPERIMENTAL

Starting Materials

The urea used was commercial material previously dried at 110° C. Ammonium chloride used was Merck Reagent. Aluminum sulphate was the anhydrous material which had been heated at 500° C.

Procedure

An intimate mixture of finely powdered urea, ammonium chloride, and aluminum sulphate was placed in a glass liner. Enough ammonia (liquid) was added to the mixture to build the desired pressure. After the bomb was closed and the necessary connections were made, the reaction mixture was heated to the temperature of synthesis and in most cases vented to 1000 p.s.i.g. The period of heating was recorded from the time when the temperature of reaction was attained. After the mixture was cooled, the solid products were boiled with ammoniacal water. Aluminum hydroxide was filtered off and the filtrate was analyzed for guanidine and melamine contents.

Identification of Guanidine

(*A*) The picrate obtained from the solution presumed to contain guanidine was treated with an excess of dilute nitric acid and boiled. Then the mixture was exhaustively extracted with benzene to remove picric acid. By evaporation of the aqueous layer, a residue was obtained, m.p. 203–205° C. Recrystallizations from ethanol raised the melting point to 212–213° C. A mixed melting point with an authentic sample of guanidine nitrate was not depressed.

(*B*) A portion of the filtrate was evaporated to dryness and the residue dissolved in the minimum amount of water. Then ammonium nitrate was added, and the solution was heated to boiling and allowed to cool. Crystals separated out, m.p. 202–205° C. Recrystallizations from ethanol raised the melting point to 212–213° C.

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SYNTHESIS OF γ-HYDROXYGLUTAMIC ACID (DIASTEREOMERIC MIXTURE)[1]

By Léo Benoiton and L. P. Bouthillier

ABSTRACT

γ-Hydroxyglutamic acid has been synthesized by two independent methods, namely the condensation of ethyl α-acetoxy-β-chloropropionate with diethyl acetamidomalonate, and the hydroxylation of glutamic acid. Chemical evidence is provided for the structure of the compound.

γ-Hydroxyglutamic acid has been proposed as an intermediate in the metabolism of hydroxyproline (3, 8). In 1941, Dakin (1) claimed to have prepared this dicarboxylic amino acid; however, the details of his synthesis were never published. As yet, there is no method available for the synthesis of this compound. We wish to report here the synthesis of γ-hydroxyglutamic acid by two independent methods.

For the first method, Fig. 1, ethyl α-acetoxy-β-chloropropionate (I) was condensed with diethyl acetamidomalonate (II) in the presence of sodium

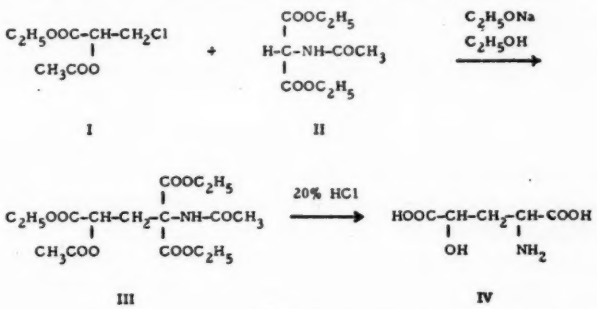

FIG. 1

ethoxide to form diethyl α-acetamido-α-carbethoxy-γ-acetoxyglutarate (III) which on acid hydrolysis gave γ-hydroxyglutamic acid (IV). The free amino acid crystallized as the monohydrate from an aqueous solution at pH 2.5. The same product was obtained by condensing diethyl acetamidomalonate with ethyl α-acetoxyacrylate instead of ethyl α-acetoxy-β-chloropropionate in the presence of a catalytic amount of sodium; however the yield was not any better.

The structure of the compound was confirmed by the fact that it is resistant to periodate oxidation, oxidized to aspartic acid by potassium permanganate, and reduced to glutamic acid by hydriodic acid. The formation of aspartic acid and glutamic acid was demonstrated by paper chromatography in several solvents.

[1]*Manuscript received June 27, 1955.*
Contribution from the Department of Biochemistry, Faculty of Medicine, University of Montreal, Montreal, Quebec.

For the second method, Fig. 2, N-phthaloyl-L-glutamic anhydride (V) was brominated and the γ-bromo-N-phthaloylglutamic acid (VI) thus obtained was hydroxylated to give γ-hydroxy-N-phthaloylglutamic acid (VII) which on hydrolysis gave γ-hydroxyglutamic acid (IV). The crystalline product obtained was contaminated with glutamic acid and could only be adequately purified by column chromatography. The pure amino acid coincided on paper

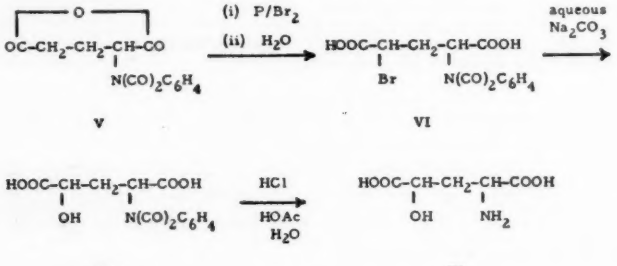

FIG. 2

chromatograms (Table I) with the γ-hydroxyglutamic acid prepared by the first method, and when submitted to the action of periodate, permanganate, and hydriodic acid, it gave results which were identical with those previously obtained.

EXPERIMENTAL*

1. *Preparation of γ-Hydroxyglutamic Acid*

(*a*) *Condensation of Ethyl α-Acetoxy-β-chloropropionate with Diethyl Acetamidomalonate*

To a solution containing 1.38 gm. (0.06 gm-atom) of sodium and 10.85 gm. (0.05 mole) of diethyl acetamidomalonate in 100 ml. of anhydrous alcohol was added dropwise 9.75 gm. (0.05 mole) of ethyl α-acetoxy-β-chloropropionate (4, 5). The reaction flask was placed on a mechanical shaker overnight and then the sodium chloride was removed by centrifugation and washed with alcohol. The combined supernatant and washings were freed of alcohol by distilling under reduced pressure and the remaining condensation product was refluxed for three hours in 100 ml. of 20% hydrochloric acid. The excess hydrochloric acid was removed by repeated distillation under reduced pressure and the residue was taken up with 25 ml. of water and decolorized with charcoal. The pH of the solution was adjusted to 2.5 with concentrated ammonia and the solution was cooled. The white crystalline mass obtained was filtered and recrystallized from water–alcohol. A further crop requiring several recrystallizations was obtained from the mother liquor by addition of alcohol. Total yield: 35%; m.p. 171–173° C. dec. Analysis for $C_5H_9NO_5 \cdot H_2O$: C, 33.89, 33.85; H, 6.23, 6.18; N, 7.81, 7.70. Calc.: C, 33.17; H, 6.12; N, 7.73.

When heated at 110° C. over phosphorus pentoxide *in vacuo*, the compound

**Analyses by the Geller Microanalytical Laboratories, Hackensack, N.J.; melting points by capillary method, uncorrected.*

loses two molecules of water to presumably give the lactam.* Analysis for $C_5H_7NO_4$: C, 42.23, 42.52; H, 4.78, 4.99; N, 9.80, 9.57. Calc.: C, 41.39; H, 4.86; N, 9.65.

(*b*) *Hydroxylation of* L-*Glutamic Acid*

N-Phthaloyl-L-glutamic anhydride was prepared by the method of Sheehan and Bolhofer (7). It was then brominated using a technique described by Eck and Marvel (2). Dry bromine, 146 gm., was added dropwise, with stirring, to a cooled mixture of 63 gm. of N-phthaloyl-L-glutamic anhydride and 9.4 gm. of red phosphorus. The mixture was then warmed on a water bath for six hours and the hot mixture was poured slowly into 500 ml. of water with vigorous stirring. The hydrolysis of the acyl bromide must be initiated by gentle warming, but it must then be controlled by cooling. The light yellow crystals obtained upon cooling were recrystallized from 500 ml. of hot water containing a few grams of bisulphite.

The product (35 gm.) was then refluxed in 250 ml. of 2 *N* sodium carbonate for three hours. The sodium ions were removed by adding sufficient Amberlite IR-120 to bring the pH down to 1, and the solution was concentrated under reduced pressure. The oily residue was refluxed for two hours in a mixture of 70 ml. of concentrated hydrochloric acid, 70 ml. of glacial acetic acid, and 70 ml. of water (6). The solution was cooled, the phthalic acid was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was taken up with 25 ml. of water, filtered, and treated successively with silver carbonate, hydrogen sulphide, and charcoal. The solution was concentrated to 25 ml. and alcohol was added. The white crystals which formed (8 gm.) were chromatographed in two portions on an 8×50 cm. column of Dowex 50 (200 to 400 mesh) in the acid form using *N* hydrochloric acid as the eluting agent. The fractions containing the γ-hydroxyglutamic acid were combined and evaporated to dryness under reduced pressure. The residue was dissolved in 25 ml. of water and the solution was adjusted to pH 2.5 and cooled. The yield was 10% based on the N-phthaloylglutamic anhydride employed. The decomposition point observed was ill-defined (about 150°), being lower than the one reported above. This might well be due to a difference in the relative amounts of the optical isomers in the products obtained by the two methods. Analysis for $C_5H_9NO_5 \cdot H_2O$: C, 34.02, 34.13; H, 6.08, 6.05; N, 7.78, 7.61. Calc.: C, 33.17; H, 6.12; N, 7.73.

2. Characterization of γ-Hydroxyglutamic Acid

(*a*) *Paper Chromatography*

Five-microgram quantities of γ-hydroxyglutamic acid dissolved in water were chromatographed on Whatman No. 1 filter paper in several solvent systems using the ascending technique. The amino acid spots were revealed by spraying the chromatograms with a 0.1% solution of ninhydrin in *n*-butanol, and by heating them at 70° for 15 min. The results appear in Table I. The gray spots obtained in the last three solvents change to purple in 24 hr.

**The ninhydrin reaction was positive only after prolonged heating of the dehydrated product in the presence of the reagent.*

TABLE I

CHROMATOGRAPHY DATA

Solvent system	R_f	Color
Phenol–water (80: 20)	0.13	Purple
n-Butanol – acetic acid – water (60: 12: 28)	0.17 and 0.19	Pink and purple
Pyridine–water (65: 35)	0.53	Gray
n-Butanol–methanol–benzene–water (2: 4: 2: 2)	0.26	Gray
n-Butanol – 95% ethanol – ammonia (8: 1: 3)	0.02	Gray

In butanol – acetic acid – water, γ-hydroxyglutamic acid gives two overlapping spots of different colors. This is most probably due to the presence of two diastereoisomers.

(*b*) *Permanganate Oxidation*

A warm solution of 50 mgm. of γ-hydroxyglutamic acid in 50 ml. of 2 *N* sulphuric acid was titrated with a solution of potassium permanganate until no more reagent was consumed. The amino acids were extracted from the solution by shaking one hour with 5 gm. of Dowex 50 (20 to 50 mesh), and then displaced from the resin with 25 ml. of 5 *N* ammonium hydroxide (9). The eluate was evaporated to dryness, the residue was taken up with water, and aliquots of the solution were chromatographed. The only amino acid present in the solution coincided with aspartic acid in the five solvent systems described in Table I.

(*c*) *Reduction with Hydriodic Acid*

Forty milligrams of γ-hydroxyglutamic acid, 50 mgm. of red phosphorus, and 3 ml. of 57% hydriodic acid were heated in an evacuated sealed tube at 150° C. for eight hours. The mixture was diluted to 30 ml. with water and the amino acids were extracted from the reaction medium as in the previous experiment. Chromatograms of the resulting solution gave only one spot which coincided with glutamic acid in the same five solvents.

(*d*) *Periodate Oxidation*

A mixture of 10 mgm. of γ-hydroxyglutamic acid in 15 ml. of water, 10 ml. of 0.5 *M* sodium periodate, and 5 ml. of *M* sodium bicarbonate was allowed to stand for one hour. Chromatograms of the desalted solution, obtained as previously described, indicated a quantitative recovery of the original amino acid.

ACKNOWLEDGMENT

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PHYSICOCHEMICAL STUDIES OF LIGNINSULPHONATES

I. PREPARATION AND PROPERTIES OF FRACTIONATED SAMPLES[1]

By J. L. Gardon[2] and S. G. Mason

ABSTRACT

High molecular weight ligninsulphonates were separated from other constituents of spent sulphite liquor by a method of dialysis allowing continuous removal of the dialyzates and their replacement by distilled water. The process was controlled by continuous analysis of the residue and dialyzate. The lower molecular weight ligninsulphonates in the dialyzates were separated from the carbohydrates by precipitating their barium salts with ethanol; four fractions corresponding to different times of dialysis were prepared in this manner. The ligninsulphonates in the dialyzed liquor were separated into four additional fractions by ultrafiltration through membranes of different pore sizes. The methoxyl, sulphur, and phenolic hydroxyl contents, neutralization equivalent weights, reducing powers, ultraviolet absorption spectra, diffusion coefficients, and number-average molecular weights of the eight fractions were determined. The molecular weights of the fractions range from 3700 to 58,000 but the integral molecular weight distribution curve indicates the presence of ligninsulphonates with molecular weights as high as 100,000.

INTRODUCTION

The dissolved solids in spent sulphite liquor consist largely of ligninsulphonates, and the remainder of sugars and acidic degradation products of cellulose and lignin. Various methods of separating ligninsulphonates from the other constituents have been used (2, 14) including precipitation, dialysis, and ion exchange. Attempts have also been made to fractionate the highly polydisperse ligninsulphonates according to their molecular weights by fractional dialysis (6, 32), fractional extraction of the free acids by propanol (31), fractional precipitation of the barium salts by ethanol (23) or acetone (25), countercurrent butanol extraction of free acids and amine salts in water (22), differential sorption on swollen ion-exchange resins (18), and fractionation by the different solubilities of various amine salts (4, 5, 19, 25).

It has been found that the methoxyl content of the fractions increases with decreasing diffusion coefficient (23), i.e. with increasing molecular weight, while the sulphur content of the fractions, with few exceptions (18), decreases with increasing methoxyl content.

Various attempts have been made to determine the molecular weights of ligninsulphonates. Cryoscopic and ebullioscopic methods (17, 30) yielded apparent molecular weights in the range of 1000 to 6000, but owing to electrolytic dissociation of the ligninsulphonates these values are probably low. Estimates of molecular weights have also been made from the diffusion coefficients (6, 7, 16) and from the dialysis rates (31) either by comparison of the ligninsulphonates with various test substances or by assuming that the ligninsulphonate molecules are spherical and non-solvated, and that the

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Contribution from the Pulp and Paper Research Institute of Canada and the Chemistry Department, McGill University, Montreal, Quebec.
[2]*Holder of the Anglo-Paper Research Fellowship and of a Studentship from the National Research Council of Canada. Present address: Industrial Cellulose Research, Ltd., Hawkesbury, Ontario.*

relation between the molecular radius and the diffusion coefficient given by the Sutherland–Einstein equation applies.

Molecular weights ranging from 2000 to 20,000 have thus been obtained. These values agree in order of magnitude with the more precise values calculated by McCarthy (22) from diffusion and sedimentation constants, and by Olleman, Pennington, and Ritter (25, 28) from diffusion and viscosity data. McCarthy (22) obtained molecular weights ranging up to 100,000.

The ultimate aim of our work was to investigate some of the basic physicochemical properties of ligninsulphonates. For this purpose relatively large quantities of chemically well-defined ligninsulphonate fractions were prepared by a combination of fractional dialysis and ultrafiltration of a spent sulphite liquor concentrate. The flow sheet of this procedure is presented in Fig. 1.

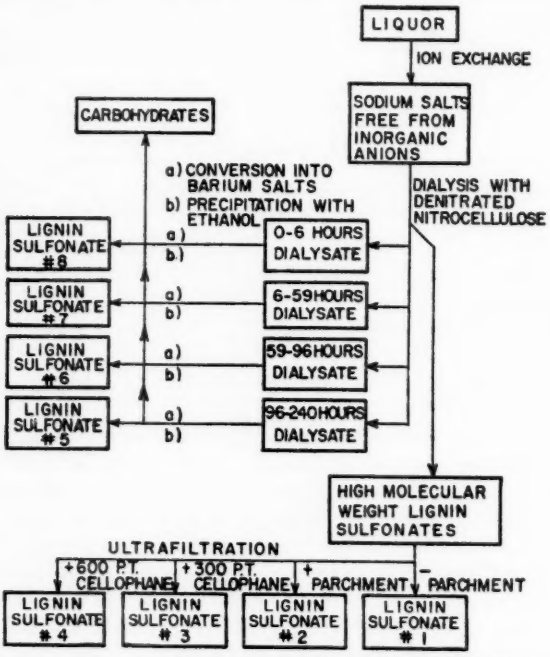

FIG. 1. Flow sheet for the fractionation of ligninsulphonates.

In this paper the fractionation techniques are described and the correlation between the analytical data and molecular weights of the samples is discussed. A number of other properties of the fractions are described in the following paper (10).

EXPERIMENTAL

Material

Spray-dried spent sulphite liquor marketed under the name of Lignosol B and supplied by Lignosol Chemicals Ltd. was used as the starting material.

The spent liquor was drawn from cooks of a mixture of balsam and spruce in approximately equal amounts.

About 700 gm. Lignosol was dissolved in 2 liters of water, filtered, passed through previously regenerated and well-washed Amberlite IR-4B (OH) anion- and IR-120 (H) cation-exchange resins to remove mineral acids and calcium respectively, and finally neutralized to pH 6.5 with sodium hydroxide. The resulting solution contained 633 gm. solids.

Fractionation

1. *Dialysis*

A Webcell Laboratory Model continuous countercurrent dialyzer (manufactured by Brosites Machine Corp., N.Y.) was used with denitrated nitrocellulose membranes. This dialyzer consists of 13 lucite rings, 17.5 cm. I.D. The membranes are clamped between these rings yielding seven water, and six solution chambers connected respectively in series through suitable channels. These channels were not used as it was found that the apparatus could be controlled better if the corresponding chambers worked in parallel. The design was accordingly altered by drilling holes in the top and bottom of each ring.

As shown in Fig. 2, the liquor was circulated from a reservoir through the dialyzer, with a continuous countercurrent flow of distilled water. A

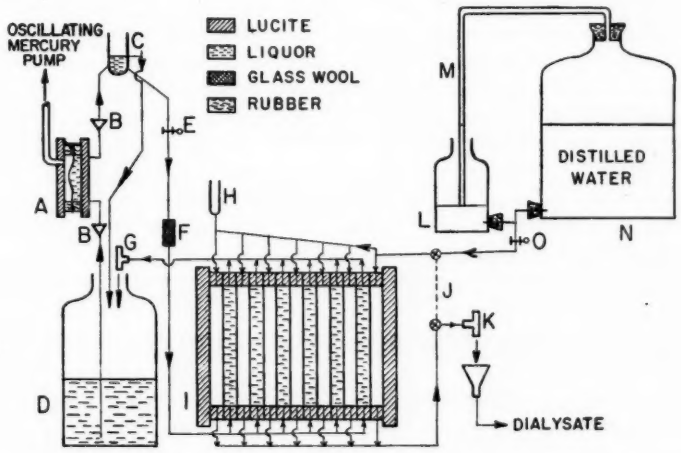

FIG. 2. The dialysis unit: (A) membrane pump, (B) valves, (C) liquor overflow, (D) liquor reservoir, (E) pinchcock to regulate liquor flow, (F) glass wool filter, (G) T tube, (H) air inlet, (I) dialysis cell, (J) bypass for initial filling of the water chambers, (K) T tube, (L) constant head device, (M) air inlet tube, (N) distilled water reservoir, (O) pinchcock to regulate water flow.

membrane pump (A) governed by an oscillating mercury pump was used for this purpose. The functioning of the pump is described in detail elsewhere (8).

To ensure steady flow the pump did not feed directly into the dialyzer, but passed through an overflow (C) and liquor flowed through the unit by gravity. The flow rate was regulated to 60 cc./min. by a pinchcock (E).

The distilled water came from a reservoir (N) and through a constant head device (L) at 3 cc./min. The free head of the water in the constant head device was maintained by the air inlet tube (M) which controlled the flow of air into and the flow of water from the reservoir (N). In this way the head of water was automatically maintained constant within 2 cm.

The volume of liquor was maintained between four and six liters. The volume increase due to osmosis amounted to about two liters per 12 hr.; this excess was periodically removed, neutralized with sodium hydroxide to avoid polymerization of the ligninsulphonic acids, concentrated by vacuum evaporation at 60°C., and reintroduced into the system.

To avoid growth of fungi during dialysis and the subsequent ultrafiltration, a small amount of toluene was added to the liquor.

The dialyzates produced every 12 hr. were handled as separate fractions. They were brought to pH 6.5 with sodium hydroxide or with cation-exchange resin as required. The content, reducing power, and neutralization equivalent weight of solids were determined. The reducing power of the solids in the residual liquor was calculated from these results by difference and checked by direct determination every 48 hr.

The dialysis was stopped after 19 days when the reducing power of the solids in both dialyzates and residual liquor became of the same order of magnitude (Fig. 3, curves F and G).

To separate the carbohydrates from the β-ligninsulphonates, i.e. the low molecular weight fractions, the barium salts were precipitated with ethanol as suggested by Erdtman (5). The dialyzates corresponding to 0 to 6, 6 to 59, 59 to 98, 98 to 240 hr. of dialysis were respectively combined to yield four separate fractions from which sodium was removed by cation exchange. The resulting free acids were neutralized with barium hydroxide, and the solutions were concentrated by vacuum evaporation at 60°C. to about 30% solid content and poured dropwise into four volumes of ethanol with vigorous stirring. The precipitates were twice dissolved in water and reprecipitated. The barium salts were passed through a cation exchanger and the free acids obtained were neutralized with sodium hydroxide and dried at 60°C. under vacuum, yielding fractions Nos. 5 to 8. The alcoholic mother liquors containing the carbohydrates were discarded; it should be noted, however, that in subsequent investigations it would be well worth while to study these constituents.

2. *Ultrafiltration*

Evidence presented later indicated that the residual liquor at the end of the dialysis contained pure ligninsulphonates. The residue was separated into four fractions (Nos. 1 to 4) by means of ultrafiltration using 600 P.T. cellophane, 300 P.T. cellophane, and No. 27 parchment membranes as supplied with the Webcell dialyzer.

It can be concluded from the results of the fractionation procedure that the

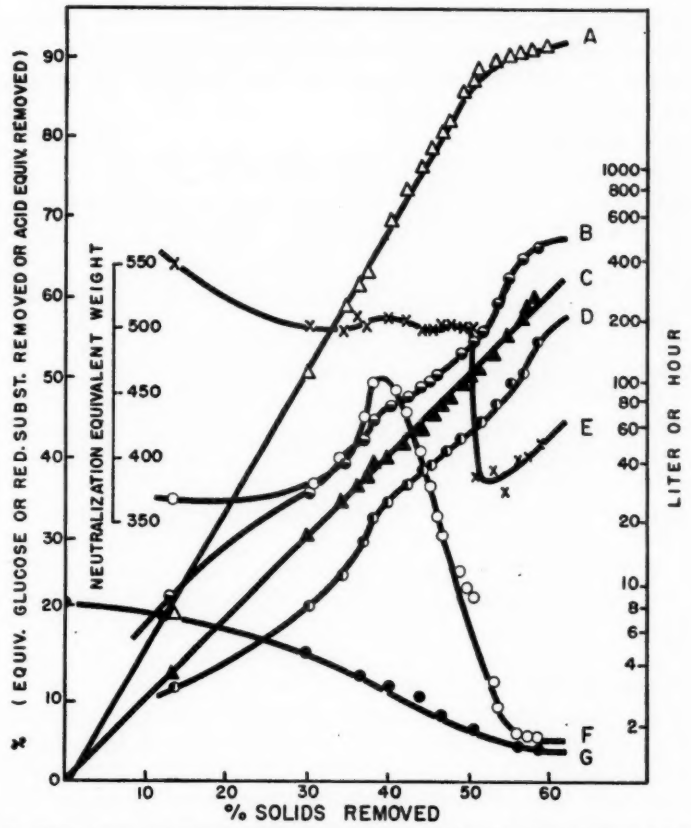

FIG. 3. Dialysis control diagrams showing per cent reducing substances removed (curve A), dialysis time (B), per cent acid equivalents removed (C), cumulative dialyzate volume (D), neutralization equivalent weight of the solids in the dialyzate (E), reducing power of the solids in the dialyzate (F), and reducing power of the solids in the dialyzed liquor (G).

denitrated nitrocellulose membranes used in dialysis have the smallest, and the parchment membranes the largest pore size.

The ultrafilter developed for the purpose of fractionating the lignin-sulphonates is described in detail in a separate publication (8); six ultrafilter cells each having a membrane area of 200 sq. cm. were used with cellophane and three with parchment membranes. The residual liquor was circulated in these cells and its volume was maintained constant at 1 liter. The ultrafiltration was stopped after 3, 7, and 12 days for the parchment, 300 P.T. cellophane, and 600 P.T. cellophane membranes respectively, the end points being determined as described later.

The ultrafiltrate of the 600 P.T. cellophane is fraction No. 4 and the fraction that did not pass the parchment is fraction No. 1. Before the solutions of the

different fractions were evaporated, they were adjusted to pH 6.5 by treatment with cation exchanger and addition of hydroxide as required.

All four of these fractions could be quantitatively precipitated with barium and alcohol.

Analytical Methods

To determine the neutralization equivalent weights, the sodium was removed by Amberlite IR-100 (H) cation exchanger and the free acid thus obtained was titrated with methyl red as indicator. Separate conductivity and pH titration experiments showed that all fractions have only one titration end point at pH 6.5. The titration curves were very similar to the ones presented by Peniston and McCarthy (26).

After the ligninsulphonates were oxidized with nitric acid the sulphur was determined as barium sulphate, as suggested by Yorston (34).

The methoxyl content was determined by the method of Peniston and Hibbert (27) adding red phosphorus, as suggested by Vjebock and Schwappach (33), instead of phenol to the hydriodic acid ligninsulphonate mixture.

The reducing power of the fraction was compared to that of glucose by means of Fehling solution and expressed as equivalent glucose, as described by Yorston (34).

Ultraviolet absorption spectra were measured in a Beckman Model DU spectrophotometer in pH 6 and pH 12 solutions with the buffers as blanks. It was shown that Beer's Law is valid for all fractions when ultraviolet light is used.

Diffusion Coefficients

The diffusion coefficients were evaluated in 0.5 *N* sodium chloride solution by the porous plate method (21, 24) in a modified apparatus described elsewhere (9). The diffusion cells were calibrated with 0.1 *N* potassium chloride, using Gordon's value (12) of 1.588 sq. cm./day for this solution. The ligninsulphonate concentrations were calculated by means of Beer's Law from the optical densities at 280 mμ.

Molecular Weights

The molecular weights were established from osmotic pressure measurements. To suppress dissociation 0.5 *N* aqueous sodium chloride was used as solvent. When necessary the results were corrected for membrane leakage as described in a separate paper (9). All data presented refer to neutral sodium salts.

RESULTS AND DISCUSSION

Dialysis

The main problem in fractionation of polydisperse materials by dialysis is to determine when the process is ended, since small amounts continue to diffuse through the membrane after long periods of dialysis. It was not possible to establish firm analytical criteria for the purity of ligninsulphonate fractions because their reducing power, sulphur content, neutralization equivalent weight, and methoxyl content change with the molecular weight. To complicate

matters further there are other acidic, reducing, sulphur-containing, and probably methoxyl-containing materials present in the liquor. These substances, mostly sugars and degradation products of cellulose and lignin, are believed, however, to have lower molecular weights than the bulk of the ligninsulphonates.

In Fig. 3 the control data of the dialysis are shown plotted against the cumulative percentage of the solids removed. Since the dialyzates contained materials with different diffusion rates, no theoretical relation could be derived for the time-dependence of the removal of the dialyzable solids. It was found that the concentration of the dialyzates was roughly inversely proportional to the dialysis time and cumulative dialyzate volume (Fig. 3, curves B and D).

The most significant curves are those of the reducing power of the solids in the dialyzate and in the residual liquor (curves *F* and *G*) and of the neutralization equivalent weights of the solids in the dialyzate (curve E). The following distinct stages of dialysis can be observed:

(1) The first 30% solids removed have a progressively increasing reducing power and decreasing neutralization equivalent weight as can be seen from curves F and G.

(2) In the next dialysis period 20% solids were removed. The reducing power of the solids in the dialyzate passed through a maximum while their neutralization equivalent weight remained constant.

(3) The reducing power and neutralization equivalent weight of the subsequent 2% solids removed decreased sharply and the neutralization equivalent weight passed through a minimum at 375. At this minimum the reducing power of the dialyzate was low, thus indicating that it contained little nonionic sugar. Thus it is fair to assume that the previous dialyzates contained acids with neutralization equivalent of 375 and less.

(4) There is evidence that after the diffusion of 52% solids, the residual liquor consists of carbohydrate-free ligninsulphonates. This is indicated by the fact that the reducing power of the solids in the dialyzate and in the rest of the liquor is roughly the same and that, with increasing time of dialysis, the neutralization equivalent weight of the dialyzate increases; the neutralization equivalent weight of the ligninsulphonates increases with increasing molecular weight.

The reducing power of the diffusing and of the residual solids was roughly equal at the end of the dialysis indicating that the reducing power of the ligninsulphonates investigated was inherent and not due to impurities. In this connection the experiments of Peniston and McCarthy (26) are of interest. These authors plotted the reducing power of dialyzed ligninsulphonates, as obtained in a continuous countercurrent dialyzer, against the reciprocal of the time of dialysis and by extrapolating their data to a value corresponding to infinite time of dialysis concluded that really pure ligninsulphonates should not be reducing. The present experiments indicate that care must be taken in such an extrapolation. The curve G can be extrapolated to zero from the region of 30 to 52% solids removal but, on the other hand, it runs almost parallel to the horizontal axis.

Degree of Fractionation

Neither fractional dialysis nor ultrafiltration as employed allow absolute separation of the different molecular weight fractions; while this is self-evident in fractional dialysis, it needs further explanation for ultrafiltration.

The behavior of solutes in ultrafiltration depends on sizes of the molecules, and the membrane pores. Materials with relatively small molecules can pass through the membrane in unchanged concentration, intermediate molecular size materials pass through the membrane with a reduced concentration, and substances with relatively large molecules are retained by the membrane.

The concentration $c_i(t)$ of a fraction i in the solution after a period of ultrafiltration t can be calculated from an equation derived elsewhere (8):

$$c_i(t) = c_i(0).e^{-\gamma_i Kt/V_0}$$

where V_0 is the volume of the residual solution, constant under the experimental conditions, K is the volume of ultrafiltrate produced in unit time, and γ_i is the partition coefficient of the solute in question between the solutions in and outside of the membrane. To determine the time of ultrafiltration it was assumed that the solution to be filtered contained a component which could pass through the membrane in unchanged concentration, i.e. γ was unity. The values of V_0 and K were determined experimentally and the time necessary for the removal of 99.5% of this component was calculated. Consequently, besides really high molecular weight substances, a portion of the materials with $0 < \gamma_i < 1$ was left behind. Thus it is to be expected that there was some overlapping in the molecular weight distributions, even for higher fractions.

Despite the imperfections in the methods of fractionation, the fractions represent well-defined molecular weight ranges. In osmotic pressure measurements it was possible to follow the diffusion rates of fractions Nos. 2 to 6 continuously with time. Furthermore, in the diffusion coefficient determinations the diffusion times were varied up to threefold and it was found that the diffusion rates were independent of diffusion time; this indicates that the fractions cannot be very heterodisperse.

Analytical Data

It has already been mentioned that the dialysis method makes it probable that the four high molecular weight fractions are carbohydrate-free ligninsulphonates; further evidence is provided by the analytical data in Table I. The acid and sulphur contents are approximately equal, thus indicating that all acid groups are sulphonic. The reducing power of these fractions is low and the methoxyl content is high.

Some 60% of the total solids of the spent sulphite liquor from which the mineral acids were removed by ion exchange can be precipitated with ethanol as barium salts. It is, however, doubtful whether this technique in itself is adequate to separate the carbohydrates from the ligninsulphonates quantitatively. If not, there may be considerable impurities present in the low molecular weight fractions.

TABLE I

ANALYTICAL DATA OF SODIUM LIGNINSULPHONATE FRACTIONS

Frac. No.	% total of original solids	% total of fractions	% methoxyl	% S	% phenolic acid	Reducing power, % eq. glucose	Neut. eq. wt.	Acid groups S / sulphur	Acid groups S / MeO	Mol. wt.	Diffusion coefficient, sq. cm./day
1	7.3	11.87	10.45	5.22	0.945	3.56	599	1.03	0.53	58000	0.0314
2	18.7	30.41	10.40	5.58	0.739	3.36	570	1.01	0.56	19200	0.0849
3	2.8	4.55	10.41	5.40	0.780	3.35	585	1.01	0.55	15500	0.113
4	10.2	16.58	9.82	5.98	0.870	3.79	535	1.00	0.64	8500	0.134
5	4.8	7.80	8.80	6.37	0.770	7.18	450	1.11	0.81	5200	0.178
6	9.5	15.45	7.24	6.25	0.696	9.20	375	1.36	0.89	4600	0.194
7	3.4	5.53	4.06	5.55	0.672	9.42	289	2.00	1.46	3700	0.219
8	4.8	7.80	2.25	5.02	0.530	8.47	227	2.81	2.38	3650	0.221
Total	61.5%	100%									

*Calculated from differential ultraviolet extinction coefficient (see text).

Fractions 5 to 8 contain more acidic groups than calculated on the basis of their sulphur content. Freudenberg *et al.* (7) found similar analytical results and suggested that these were due to the presence of carboxyl groups in the ligninsulphonates. An alternative explanation is that the carboxyl groups belong to the contaminating carbohydrates.

The sulphur contents of the fractions increase with decreasing molecular weights up to fraction No. 5 and then decrease. This peculiar drop in sulphur contents after fraction No. 5 may be caused by the presence of sulphur-free impurities.

Even if the low molecular weight fractions are contaminated, there is no doubt that their bulk is made up of ligninsulphonates chemically similar to the higher fractions. The strongest evidence is furnished by ultraviolet absorption spectra.

It is well known that the ultraviolet absorption spectra of lignin preparations in neutral solution have maxima near 280 mμ and minima near 260 mμ. Not only do all of the fractions show the same pattern (Fig. 4, Table II) but

TABLE II

CHARACTERISTIC DATA OF THE ULTRAVIOLET ABSORPTION SPECTRA

Frac. No.	pH 6						pH 12			Differential spectrum	
	ϵ_{280}, cm.$^{-1}$ gm.$^{-1}$ liter	Optical density at 280 mμ	λ_{max}, mμ	λ_{min}, mμ	$\epsilon_{max}/\epsilon_{min}$	$\epsilon_{245}/\epsilon_{280}$	λ_{max}, mμ	λ_{min}, mμ	$\epsilon_{max}/\epsilon_{min}$	λ_{max}, mμ	$\Delta\epsilon_{max}$, cm.$^{-1}$ gm.$^{-1}$ liter
1	13.9	4200	282	263	1.32	2.28	282	266	1.13	300	2.28
2	13.3	4090	283	263	1.39	2.28	281	267	1.13	298	1.78
3	13.5	4150	282	262	1.38	2.23	281	267	1.12	301	1.88
4	12.4	4020	282	262	1.29	2.20	281	268	1.07	298	2.10
5	11.1	4100	283	263	1.39	2.15	281	268	1.09	299	1.86
6	8.8	3900	283	262	1.38	2.05	282	270	1.10	300	1.68
7	5.8	4200	277	261	1.34	1.89	281	270	1.16	299	1.62
8	4.2	6360	277	258	1.30	1.59	282	273	1.09	297	1.28

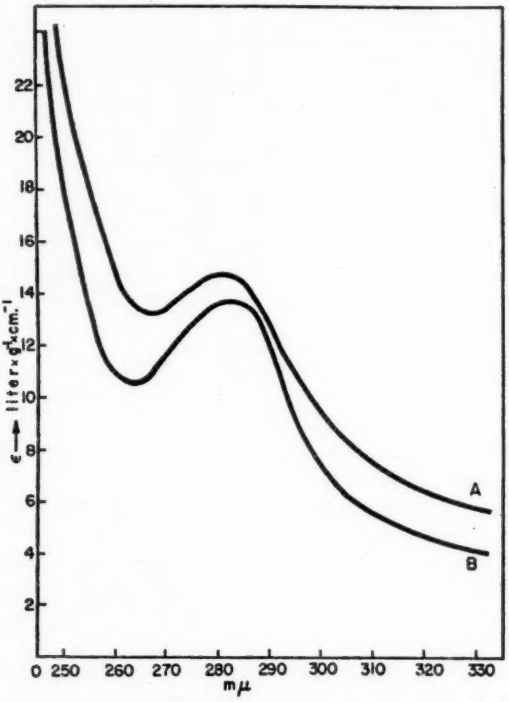

FIG. 4. Ultraviolet absorption spectra of fraction No. 1; curve A: pH 12, curve B: pH 6.

the ratio of the extinction coefficients (ϵ) at the maximum and minimum is approximately constant at 1.35 for all fractions. The only difference in the shapes of the curves is that the slopes in the lower wavelength region decrease with the molecular weights of the fractions. The ratios of extinctions at 245 and 260 mμ decrease from fraction No. 1 to fraction No. 8 (Table II).

The light-absorbing power decreases with the molecular weights of the fractions; this can be noticed by the visual observation of the color and is expressed quantitatively by the extinction coefficients for 0.1% solutions at 280 mμ (Table II). Nevertheless the ultraviolet light absorbing power per methoxyl bearing unit at 280 mμ is the same for all the fractions except the lowest, for which it is relatively high (Table II).

Aulin-Erdtman (1) found shifts in the characteristic ultraviolet spectra of lignin compounds when determined in neutral and in alkaline solutions, and attributed them to the ionization of the phenolic hydroxyl groups. The minima of the samples shifted from 263 to 266 mμ for the highest, and from 258 to 273 mμ for the lowest fraction. The values of λ_{max} decreased on the average by 1 mμ. The ratio $\epsilon_{max}/\epsilon_{min}$ was the same for all fractions, the mean value being 1.11, i.e. less than for neutral solutions.

Goldschmid (11) suggested that the phenolic hydroxyl contents of lignin

and analogous model compounds can be determined from the differential spectra of solutions with pH 6 and pH 12. These spectra were shown to have maxima around 250 and 300 mμ. With model substances Goldschmid determined the characteristic maximum extinction at 300 mμ ($\Delta\epsilon_{max}$) for 1 mole phenolic hydroxide per liter. According to this value the unknown phenolic hydroxyl content of a substance can be calculated from the following formula:

$$\%(\phi-OH) = (17/41)\ \Delta\epsilon_{max}.$$

The hydroxyl contents of the fractions (Table I) were determined from such differential spectra, characteristic data of which are shown in Table II and three of which are plotted in Fig. 5.

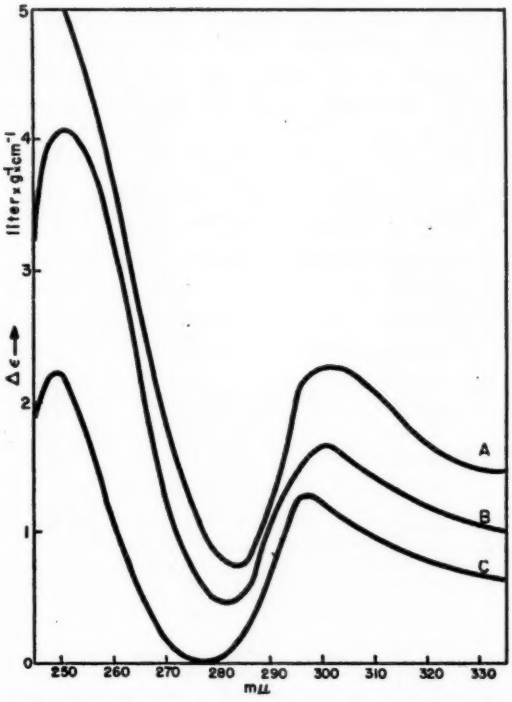

FIG. 5. Differential absorption spectra between solutions at pH 12 and pH 6; curve A: fraction No. 1, curve B: fraction No. 6, and curve C: fraction No. 8.

Molecular Weights

As described in a separate paper (9), molecular weights of all but the two lowest fractions were determined by the osmotic pressure method; these values are tabulated in Table II. When the logarithm of the molecular weight is plotted against the logarithm of the diffusion coefficient, the points corresponding to fractions Nos. 4, 5, and 6 fall on a straight line which can be extra-

polated to the measured diffusion coefficients of the lowest fractions, from which the molecular weights were calculated. It must be borne in mind that the ultraviolet extinction coefficients of the solutions were used to determine the concentrations in the diffusion experiments. Should the lower fractions be mixtures of ultraviolet light-absorbing ligninsulphonates and relatively transparent carbohydrates, the diffusion coefficients would be indicative of the ligninsulphonates alone.

In a homologous polymer series the following relation holds between the weight-average molecular weights M and diffusion coefficients D_1 (10):

$$D_1 = K\,M^{-b}$$

where b is a constant depending on the shape of the molecules and K is a constant characteristic for the homologous series. The error involved in using number-average molecular weights and the diffusion coefficients for estimating the molecular weights of the lowest ligninsulphonates should be small. As can be seen from the integral molecular weight distribution plot (Fig. 6) the degree of polydispersity of the fractions except the highest (No. 1) is relatively low. Hence there is only a slight difference between the weight-average and the number-average molecular weights.

The integral molecular weight distribution plot (Fig. 6) indicates that the

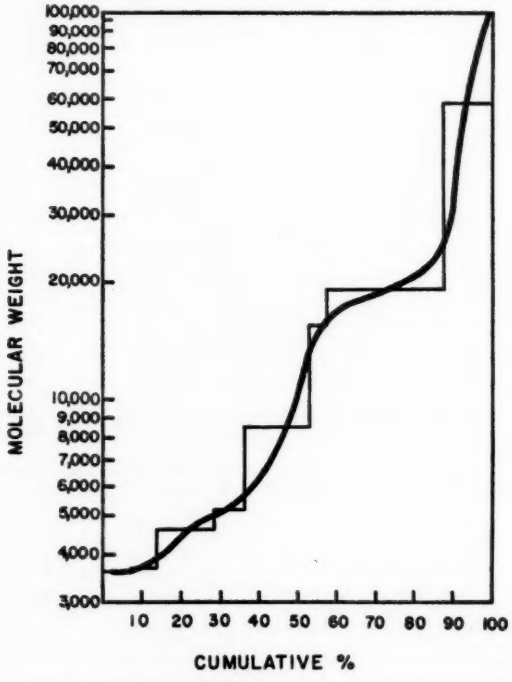

FIG. 6. Integral molecular weight distribution of the ligninsulphonates.

molecular weights of the ligninsulphonates investigated range from about 3700 to about 100,000. Around 30% of the material have molecular weights between 3700 and 5000 and also between 15,000 and 25,000.

It is beyond the scope of this paper to give a critical survey of the numerous attempts to determine the molecular weights of different lignin preparations. The accumulated data (2, 14) present a confusing picture because in the isolation of lignin or ligninsulphonates the original lignin in the wood can be considerably degraded or polymerized. Furthermore it is also possible that in the lower molecular weight region association may occur in the solutions (10); if this occurs the molecular weights obtained for the lower fractions would also be too high.

Nevertheless it is interesting to note that some investigators (3, 13, 20, 29) have found that lignin preparations isolated under mild conditions have molecular weights not lower than 3500 to 4000 which correspond well to the lower limit of the data presented here. According to Gralen (13) there is some indication that lignins with degrees of polymerization corresponding to the molecular weights 4000 and 8000 are the most stable. In the light of this, it is significant that 30% of our fractions have molecular weights in the 4000 range.

Considerably lower molecular weights of lignin, however, also have been reported (2, 15). Until a study is made of how the degree of polymerization of lignin is changed in the various isolation techniques it will be difficult to interpret the available data. Since the highest reported native lignin molecular weight is 27,000 (13), there is strong indication that during the sulphite cook at least part of the lignin becomes considerably polymerized.

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PHYSICOCHEMICAL STUDIES OF LIGNINSULPHONATES

II. BEHAVIOR AS POLYELECTROLYTES[1]

By J. L. Gardon[2] and S. G. Mason

ABSTRACT

Conductivity, dyestuff adsorption, and viscosity measurements on aqueous solutions of fractionated ligninsulphonate samples having various molecular weights indicate that they behave as flexible polyelectrolytes. There is evidence that ligninsulphonates of molecular weight less than 5000 associate in solution in a manner analogous to micelle formation in colloidal electrolytes. From the variation of intrinsic viscosity with molecular weight, it may be concluded that the degree of molecular branching of the high molecular weight ligninsulphonates is greater than that of the low molecular weight fractions.

INTRODUCTION

In the pulping of wood by the sulphite process, large quantities of lignin-sulphonic acids and salts appear in the spent liquor. Relatively large amounts of ligninsulphonate concentrates are now used as dispersants and adhesives (16, 26), although the mechanisms underlying these applications are not understood. The purpose of the investigation outlined in this paper and in subsequent papers was to study some of the physicochemical properties of the material which may be relevant to these applications.

While the exact structure of ligninsulphonates is not known, it is believed (2, 14, 16) that they are built of guaiacyl–propyl units with the sulphonate groups attached to the aliphatic chains. This molecular structure and the results of the experiments described in this paper indicate that the ligninsulphonates can be considered as substances consisting of flexible chain molecules with ionizable groups attached to them, i.e. they are polyelectrolytes.

Recently considerable research has been carried out on polyelectrolytes, general accounts of which are given by Fuoss (10, 11), Flory (8), and Katchalsky (20). The most important characteristic is that in solution the molecular shape depends upon the net electrical charge of the molecule. In the uncharged state the molecule curls up. If the ionic groups are dissociated, the polyelectrolyte molecule extends owing to the electrostatic repulsion between neighboring groups.

The degree of ionization of the polyelectrolyte depends on its concentration and also upon the presence of simple electrolytes in the solution. At low polyelectrolyte concentrations, the molecules occupy only a small part of the available space in the solution; this favors the escape of the counter-ions, e.g. the sodium ions of the ligninsulphonates, from polymer molecules, leaving the latter charged. The equilibrium between the counter-ions associated with the polymer molecules and in the part of the solvent free of polymer molecules, i.e. in the free space, is analogous to a Donnan-type equilibrium. Consequently

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when the free space becomes smaller as the polyelectrolyte concentration is increased, the counter-ion concentration in this free space greatly increases, inhibiting further ionic dissociation. The polyelectrolyte molecules can also lose their charge, even at low concentrations, if the concentration of the counter-ions in the free space is increased by the addition of simple electrolytes containing the same counter-ion as the solution.

The dependence of the charge of the molecules of the polyelectrolyte on its concentration can be shown by conductivity measurements. As the molecules progressively lose their charge with increasing concentration, less and less current can be carried by each molecule. The specific conductivity/concentration (or reduced specific conductivity) decreases with increase of the square root of the concentration, but not linearly, as predicted by the Onsager equation for simple electrolytes. The corresponding plot is curved and is steep at low concentrations.

The flexible coiling of the molecules as they progressively lose their charge can be demonstrated by the dependence of the reduced viscosity (η_{sp}/c) on the concentration. As the effect of an extended molecule on the viscosity of the solution is greater than that of a molecular coil, the reduced viscosity of the polyelectrolyte decreases with increasing concentration. If, however, determinations are made of the viscosities of solutions containing different amounts of polyelectrolyte and always the same sufficiently large amount of simple electrolyte, the reduced viscosity of the polyelectrolyte, like the viscosity of uncharged polymers, increases linearly with concentration.

In the present paper, results of conductivity, dyestuff adsorption, and viscosity measurements carried out with ligninsulphonates of various molecular weights are presented. The method of preparation of these fractions, their analytical data, their diffusion coefficients D_1, and the determination of their molecular weights by osmotic pressure measurements are reported elsewhere (12, 13).

CONDUCTIVITY MEASUREMENTS

From the foregoing considerations variation of the reduced specific conductivity with concentration of ligninsulphonates may be expected to differ from that of simple electrolytes. This apparent anomaly has been observed but not adequately explained by a number of investigators (7, 22, 27, 30). The results obtained by Samec and Ribaric (30) and Koenig (22) did not conform with the pattern predicted for polyelectrolytes and, oddly enough, the reduced specific conductivity curves had maxima at very low concentrations.

The variation of the reduced specific conductivities of several sodium ligninsulphonate fractions with $\sqrt{c}$ at 25°C. is shown in Fig. 1. Curves A and B represent fractions having molecular weights of 58,000 and 19,200 respectively and are typical of polyelectrolytes. Curves C, D, and E obtained for fractions with molecular weights 5200, 3700, and 3650 show apparent breaks; these breaks may indicate association, as generally observed (1, 25) for colloidal electrolytes, but not for polyelectrolytes. The reduced specific conductivity vs. $\sqrt{c}$ plot for a colloidal electrolyte shows a sharp discontinuity at the critical micelle formation concentration. At lower colloidal electrolyte concentrations,

the plot is linear and descends slightly; at higher concentrations, it drops very markedly and quite often passes through a minimum (1, 25). The molecular weights of colloidal electrolytes, however, are in the range of several hundreds and about 20 to 200 molecules associate to form a micelle; abrupt changes are therefore shown in the properties at the critical concentration of micelle formation. Considerably higher molecular weights were found for the

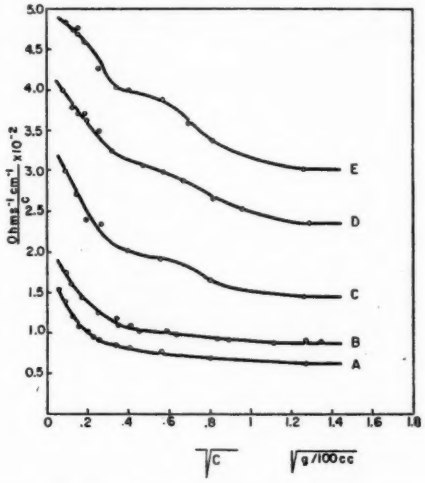


FIG. 1. Reduced specific conductivities of sodium ligninsulphonate fractions having molecular weights 58,000 (A), 19,200 (B), 4600 (C), 3700 (D), and 3650 (E).

ligninsulphonates investigated than for conventional colloidal electrolytes. Consequently it is improbable that aggregates consisting of a great number of ligninsulphonate molecules are formed. Hence the ligninsulphonate solutions do not show such sharp discontinuities as the solutions of colloidal electrolytes around the critical micelle formation concentration. The shape of curves C, D, and E may be considered as evidence that the low molecular weight ligninsulphonates are intermediate between polyelectrolytes and colloidal electrolytes. As will be shown, the reduced viscosity curves of all fractions and the dyestuff adsorption curves of all but the lowest molecular weight fraction were smooth. Hence the conductivity curves cannot be considered as absolute proof for association in the low molecular weight range; this matter requires further study.

DYESTUFF ADSORPTION MEASUREMENTS

It has been observed (15) that the optical absorption spectra of aqueous solutions of certain dyestuffs shift in the presence of micelles of colloidal electrolytes. According to Corrin and Harkins (4) this is due to adsorption or incorporation of the dyestuff on, or into, the micelle and to the different colors of the dyes in a polar medium, e.g. water, and in a non-polar medium,

as the non-charged micelle. They suggested the use of this phenomenon to detect the critical micelle formation concentration.

The influence of uncharged coiled polyelectrolyte molecules on the color of indicator dyes has not yet been investigated. The experiments here described indicate that, as was to be expected, they changed the absorption spectra of such dyes just as the micelles of the colloidal electrolytes.

Pinacyanole is blue when dissolved in pure water and green in the presence of uncharged aggregates (4). This cationic dyestuff was found to be suitable for experiments with ligninsulphonates. In Fig. 2 light-absorption curves of

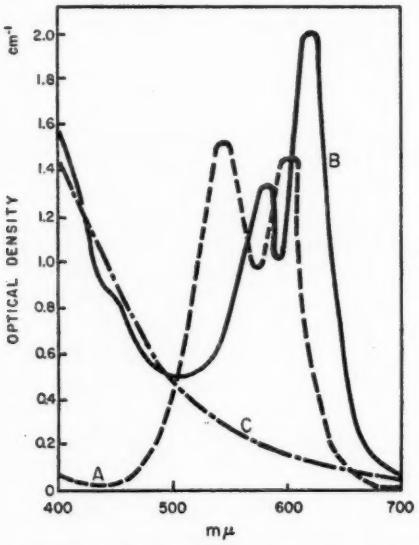


FIG. 2. Absorption curves of (A) 1.2 mgm./100 cc. pinacyanole, (B) 1.2 mgm./100 cc. pinacyanole and 0.5 gm./100 cc. ligninsulphonate (molecular weight 19,200), and (C) 0.5 gm./100 cc. ligninsulphonate.

solutions containing 1.2 mgm./100 cc. dye (A), the same amount of dye and 0.5 gm./100 cc. ligninsulphonate (B), and 0.5 gm./100 cc. ligninsulphonate alone (C) are shown. The maximum at 620 mμ in curve B is characteristic of the dyestuff – discharged aggregate complex, but the pure dyestuff solution also absorbs light at this wavelength appreciably. However, the light absorption of both pure dye and pure ligninsulphonate at 630 mμ wavelength is negligibly small. The optical density at 630 mμ can be considered as a measure of the amount of discharged aggregate – dye complex present in the solution.

The optical densities of solutions containing a fixed amount of dyestuff at various concentrations of ligninsulphonates, measured in a Beckman Model DU spectrophotometer at 630 mμ, are shown in Fig. 3.

As the molecules progressively lose their charge with increasing concentration, they adsorb increasing amounts of dyestuff. The high molecular weight

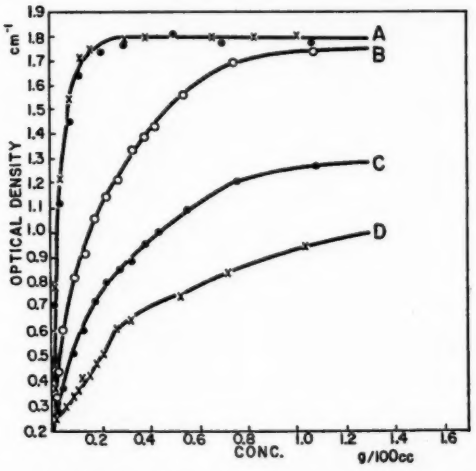


FIG. 3. Optical densities at 630 mμ of solutions containing 1.2 mgm./100 cc. pinacyanole and various amounts of ligninsulphonates. The molecular weights of the ligninsulphonates are 58,000 and 15,500 (A), 4500 (B), 3700 (C), and 3650 (D).

fractions adsorb all the dyestuff present in the solution at around 0.2% concentration. The shape of Curve A representing fractions with molecular weights 58,000 and 15,500 corresponds well to the pattern demonstrated by the conductivity and viscosity measurements.

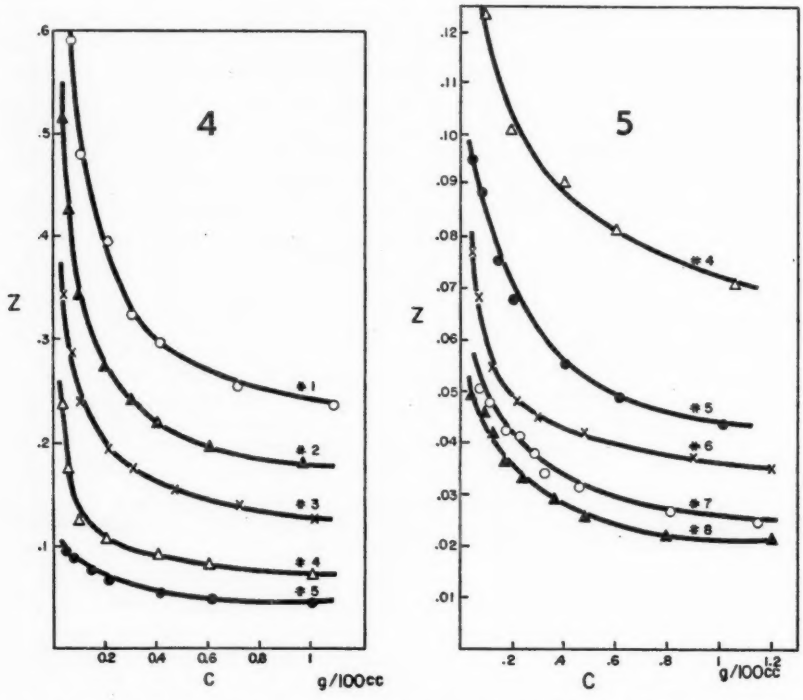
The curves B, C, and D indicate that the ability of the lower molecular weight fractions to adsorb dye is smaller than that of the high molecular weight fractions. The uncharged particles of the low molecular weight fractions are probably smaller than those of the high molecular weight fractions and, if they contain associated molecules, the number of the molecules per aggregate is probably small. The break in curve D may indicate that lowest molecular weight fractions associate in solution.

VISCOSITY MEASUREMENTS

The viscosities were determined at 25°C. in Ostwald–Cannon type (3) viscometers with flow times for water of about 400 sec. The kinetic energy corrections were negligible.

The dependence of the reduced viscosities of sodium ligninsulphonate fractions on concentration in distilled water is shown in Figs. 4 and 5. The shape of these curves is typical for polyelectrolytes; at low concentrations the reduced viscosities are high, as the molecules are extended, and at high concentrations they are low, as the molecules are coiled. The dependence of the reduced viscosities of the sodium ligninsulphonates does not follow the empirical equation of Fuoss (9):

$$[1] \qquad z = \eta_{sp}/c = [A/(1+B\sqrt{c})]+D.$$



FIGS. 4 and 5. Reduced viscosities z at various concentrations of sodium ligninsulphonates in water. The molecular weights of the number fractions are given in Table I.

In the above equation, z is the reduced viscosity, c the concentration, B a constant depending on the dielectric properties of the solvent, $(A+D)$ is the intrinsic viscosity of the polyelectrolyte when the molecules are extended, and D is a measure of the intrinsic viscosity of the randomly coiled molecules. According to this equation, the $1/(z-D)$ vs. $\sqrt{c}$ plot should be linear, but a linear plot could not be obtained for ligninsulphonates. If, however, the term $c^{\frac{1}{2}}$ was replaced by c^x in equation [1],

$$[2] \qquad z = [A/(1+Bc^x)]+D,$$

linear plots of $1/(z-D)$ vs. c^x were obtained for the three highest molecular weight fractions (Fig. 6). D was taken to be the reduced viscosity of a 4% solution; the error in doing so was very small as the reduced viscosities of solutions with concentrations higher than 2% were practically constant. The constant x was established by trial and error. With an approximate value of A, $\log[1/(z-D)-1/A]$ was plotted against $\log c$. The value of A was so chosen that this plot yielded a straight line, the slope of which was x. When D and x were known, the curves of Fig. 6 could be plotted. The intercepts on the vertical axes obtained by an analytical extrapolation were equal to $1/A$.

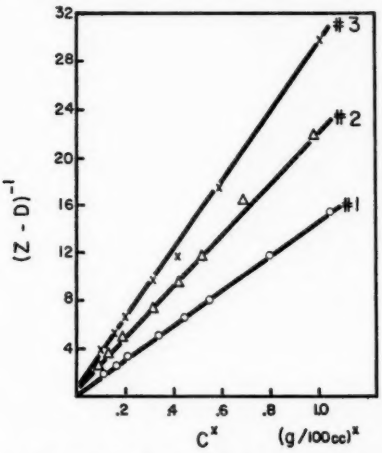


FIG. 6. $1/(z-D)$ vs. c^x plots for the three highest molecular weight fractions.

TABLE I

VISCOSITY DATA, DIFFUSION COEFFICIENTS, AND NUMBER-AVERAGE MOLECULAR WEIGHTS OF THE SODIUM LIGNINSULPHONATE FRACTIONS

		Fractions							
	Solvent	1	2	3	4	5	6	7	8
Molecular weight, gm.	0.5 *N* NaCl	58000	19200	15500	8500	5200	4600	3700	3650
Diffusion coefficient, cm./day	0.5 *N* NaCl	0.0314	0.0849	0.113	0.134	0.178	0.194	0.219	0.221
D, 100 cc./gm.	Water	0.172	0.134	0.0905	0.0563	0.0389	0.0328	0.0242	0.0211
$[\eta] = A+D$, 100 cc./gm.	Water	4–7	1.35–1.6	0.8–1.2	—	—	—	—	—
x	Water	0.71	0.73	0.78	—	—	—	—	—
$[\eta]$, 100 cc./gm.	2 *N* NaCl	0.0455	0.0425	0.0394	0.0262	0.0235	0.0220	0.0197	0.0190
k	2 N NaCl	4.9	5.0	5.42	6.00	4.75	4.15	4.6	4.6

Since this extrapolation was uncertain, the values of A thus obtained were not precise; values of $(A+D)$ are estimated to lie within the limits given in Table I. The values of x and D are also tabulated.

Equation [2] is empirical and does not have the theoretical significance of equation [1]. It is probable that the proportionality between $1/(z-D)$ and c^x is fortuitous and that the true equation describing the dependence of the reduced viscosity of the ligninsulphonates on their concentration is that of Schaefgen quoted by Katchalsky (20):

$$z = [A/(1+B\sqrt{c}+B'c)]+D.$$

The concentration dependence of the reduced viscosities of the other fractions cannot be expressed by equation [2]. Thus the intrinsic viscosities of the extended molecules could not be determined. The D values determined at a 4% concentration are given in Table I.

The reduced viscosities of the fractions in 2 N sodium chloride are shown in Fig. 7. The concentration dependence of the reduced viscosities can be

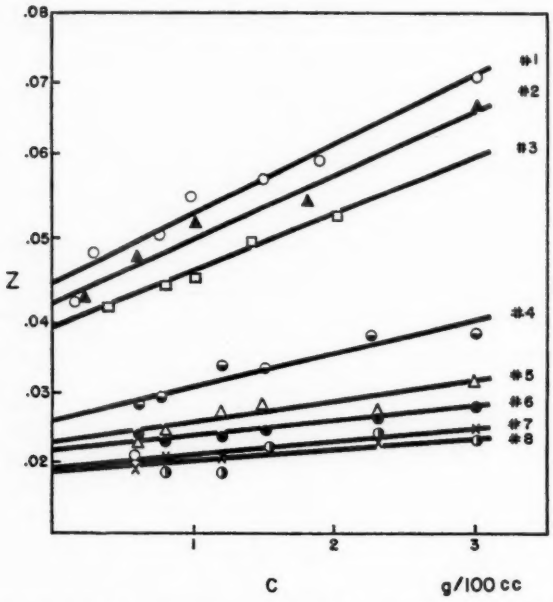


FIG. 7. Reduced viscosities at various concentrations of sodium ligninsulphonates in 2 N sodium chloride. The molecular weights of the various fractions are listed in Table I.

expressed by the equation of Simha (32) and Huggins (19) derived for non-charged interacting particles:

$$z = [\eta]+k[\eta]^2c+ \ldots \quad .$$

In this equation $[\eta]$ is the intrinsic viscosity and k is a constant characteristic for the homologous polymer series. Both $[\eta]$ and k are given for all fractions in Table I. As can be seen k changes between 4.2 and 6. Ligninsulphonates are approximately, but not truly, members of a homologous series and this may account for the variation of k.

RELATIONS BETWEEN INTRINSIC VISCOSITIES, DIFFUSION COEFFICIENTS, AND MOLECULAR WEIGHTS

According to the theories of Kuhn (23), Kirkwood and Riseman (21), and Debye and Bueche (5, 6), the constants a and b in the equations

$$[\eta] = K_2 M^a,$$
$$D_1 = K_1 M^{-b}$$

describing the dependence of the intrinsic viscosities $[\eta]$ and the diffusion coefficients D_1 on the molecular weights are both equal to unity, if the molecules are linear flexible chains, randomly coiled, and permeable to the solvent, and are equal to 0.5, if the randomly coiled flexible molecules are impermeable to the solvent. The constants K_1 and K_2 are characteristic of the homologous series. Kuhn and Kuhn (24) showed that these theories are also applicable to branched molecules if these chains are flexible and randomly coiled. The intrinsic viscosities of branched-chain molecular structures are lower than those of linear molecules of comparable size. Huggins (17, 18) has shown that $a = 2$ when the molecules behave as rigid rods.

It has been experimentally verified for a number of polyelectrolytes (20) that the values of a for the intrinsic viscosities $(A+D)$, corresponding to the extended molecules, approach 2 if the polyelectrolytes are linear, and are higher than unity but less than 2 if the polyelectrolytes are branched (31). As mentioned before, D is a measure of the intrinsic viscosity of a randomly coiled polyelectrolyte. Depending on the degree of ionization, D changes linearly with, or proportionally to the square root of, the molecular weight (20).

The diffusion coefficients, the D values, and the intrinsic viscosities in 2 N sodium chloride are plotted on a logarithmic scale against the number-average molecular weights of the ligninsulphonate fractions (Fig. 8). From the slopes

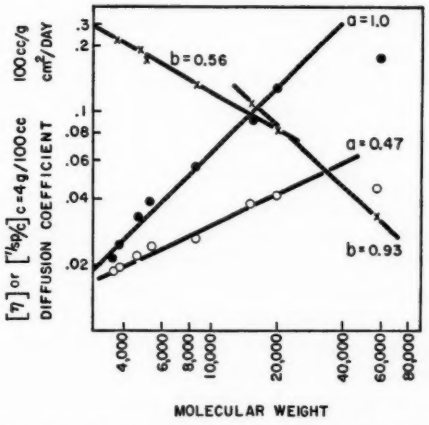


FIG. 8. Determination of constants a and b from reduced viscosities at 4% sodium ligninsulphonate in pure water (solid circles), intrinsic viscosities in 2 N sodium chloride (open circles), and diffusion coefficients in 0.5 N sodium chloride (crosses).

of these plots it can be concluded that $a = 1$ for the molecular weight dependence of D and 0.47 for the intrinsic viscosities in 2 N sodium chloride if the highest molecular weight fraction is disregarded. According to Debye (6)

the intrinsic viscosity is a measure of the true partial specific volume. As can be seen, the intrinsic viscosities in salt solution are smaller than the D values. Consequently in the sodium chloride solution, probably owing to the osmotic pressure of the sodium chloride, the coils are compressed, while in pure water the coils are expanded. The compressed coils are impermeable to water, the loose coils are permeable, and hence the values of a are in the range of 0.5 and 1 respectively.

Both the intrinsic viscosity in 2 N sodium chloride and the D value of fraction No. 1 are lower than predicted by the regularities described above. As shown in a previous communication (12), this fraction is more heterodisperse than the others and probably contains ligninsulphonates with molecular weights as high as 100,000. It is fair to assume that the higher the degree of polymerization, the greater the probability of branching during polymerization in the sulphite cook. If so, the reason for the relatively low D and low intrinsic viscosity of the highest molecular weight fractions is its higher degree of branching.

The diffusion coefficients were determined in 0.5 N sodium chloride (13). It seems that there are two b values describing the dependence of the diffusion coefficients on the molecular weight: $b = 0.56$ corresponds to all fractions but Nos. 1 and 3, while $b = 0.93$ corresponds to fractions Nos. 1, 2, and 3; this requires further investigation.

In connection with these experimental results, it is of interest to mention the work of Olleman, Pennington, and Ritter (28), who determined the diffusion coefficient, intrinsic viscosity, and partial specific volume of an ammonium ligninsulphonate in 1 N ammonium acetate solution. Using the Polson–Kuhn equation (29), they calculated the axial ratio to be 4.6. The weight-average molecular weight 21,000 calculated by them for a ligninsulphonate with 0.080 sq. cm./day diffusion coefficient compares favorably with the diffusion coefficient 0.0847 and number-average molecular weight 19,200 of fraction No. 2.

CONCLUSIONS

The results presented above lead to the conclusion that ligninsulphonates are flexible polyelectrolytes. At high concentrations of ligninsulphonates, and at low concentrations when sodium chloride is present, the molecules are coiled. At low ligninsulphonate concentrations and in the absence of sodium chloride the molecules become extended. There are indications that the low molecular weight fractions associate in solution and that the molecules of the highest molecular weight fraction are more branched than those of the other fractions. It is probable that the dispersive and adhesive properties of ligninsulphonates are due to their polyelectrolyte nature.

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STUDIES ON ALUMINA CATALYSTS

IV. THE PRODUCTION OF DIETHYLANILINE BY CONDENSATION OF ETHANOL AND ETHYLANILINE[1]

By L. A. Munro and R. A. Washington

ABSTRACT

The condensation of ethanol and ethylaniline was studied using alumina catalysts at 300°C. The gel water of the alumina affects its catalytic activity. Maxima and minima in activity occur at gel water values corresponding to those previously found for the dehydration of ethanol and formic acid. There is no concurrent change in crystal structure of the alumina gel. The results suggest the initial dehydration of alcohol to ether followed by condensation of the ether and ethylaniline to give diethylaniline. It is concluded that the best and poorest spatial arrangement of 'active points' or adsorption spaces is established by the amount of residual gel water.

In general, heterogeneous catalysis is pictured as involving adsorption of the reactants on the surface of the catalyst with activation of the substrate followed by reaction and removal of the products. The patent literature gives ample evidence of the specific and distinctive behavior of catalysts differing in chemical nature, e.g. metals such as nickel for hydrogenation and hydrous oxides and phosphates for dehydration. Many workers have found that even in the same type of catalysts there are not only differences in over-all activity but also in specificity. It is to be expected that one or both of these qualities may be affected by the size and valence of the metallic element present, by alteration of the crystal lattice, and by changes in porosity or total surface.

Activation of a hydrous oxide consists in the removal of the major portion of the gel water by heating. The temperature used in activation is cited repeatedly as a criterion of activity. In the case of alumina catalysts for the production of ether "optimum activation temperatures" from 310° to 600°C. have been reported by various authors. Of fundamental importance is the degree of removal of gel water and the crystal structure of the colloidal fibrils with concurrent alteration of the surface.

It has been found that alumina catalysts prepared by different workers in this laboratory at intervals of several years showed maxima (or minima) in activity at the same gel water irrespective of the temperature of activation (1, 9, 10). Furthermore, in the dehydration of formic acid and the production of ether and ethylene from ethanol, these maxima and minima were not due to changes in crystal lattice (9).

The present paper reports a similar study of the effect of gel water on the condensation of ethanol and ethylaniline to give diethylaniline.

This investigation was not concerned with the determination of conditions of temperature and space velocity which would give 100% conversion of ethylaniline to diethylaniline. Conditions were chosen such that the production of diethylaniline would be sufficiently high that variations in yields could be readily measured, while side reactions such as the dehydration of the ethanol

[1]*Manuscript received June 17, 1955.*
Contribution from the Department of Chemistry, Queen's University, Kingston, Ontario.

to ether and ethylene would be depressed as much as possible. A reaction temperature of 300°C. was chosen. At this temperature no appreciable change in the activation of the catalyst would occur.

The apparatus was essentially similar to that used in the previous work (9) except that the catalyst tube was half filled with pyrex wool. This acted as the vaporization chamber for the reaction mixture (1 mole ethylaniline to 1.05 moles ethanol). Reagents were analar grade. The ethylaniline was redistilled at 10 mm. The refractive index of the product was 1.5422 (n_D^{20}). The product was also analyzed for total and tertiary amines by the method of Siggia, Hanna, and Kervenski (12). The results showed the ethylaniline to be free of tertiary amines, and to have a total amine content of 8.27×10^{-3} M./gm. If the figure given in the literature is accepted (8.24×10^{-3} M./gm.) this would indicate a purity of 99.4%. The absolute alcohol tested 99.8%. The catalysts were prepared from a combined batch of alumina gel as described previously (9). The air-dried gel contained 36.7% water.

Reaction products were collected in a cold receiver and gaseous products in a gasometer over saturated brine at atmospheric pressure. The liquid products were allowed to separate into aqueous and amine layers and these analyzed. Diethylaniline was determined by an adaptation of the colorimetric method of English (5) using a Klett-Summerson photometer. The standardization curve was prepared by using a series of samples containing known mixtures (by weight) of carefully purified mono- and di-ethylaniline.

Analysis of the gaseous products indicated almost pure ethylene. Ether was detected in the liquid products but the amounts were so small that they have been neglected in calculating the materials balance, which with one exception is over 90, the average being 96.3%.

The apparatus was swept out with nitrogen before and at the conclusion of each run. A blank run with the catalyst tube packed with pyrex wool gave no ethylene, ether, or diethylaniline at 300°C.

TABLE I

Catalyst	G	M	K	L	P	N	J	I	O	R	Q
Gel water, %	9.4	7.0	6.6	6.2	5.2	4.5	3.8	3.5	1.8	0.5	0.0
% EtOH–$PhNEt_2$	13.7	15.0	17.3	15.0	25.0	16.6	22.8	22.1	17.2	22.1	22.9
% PhNHEt–$PhNEt_2$	14.9	15.7	18.1	15.7	26.1	17.3	23.8	23.0	18.0	23.1	23.9
% EtOH–C_2H_4	12.9	13.6	31.0	13.9	26.9	13.3	17.4	29.7	14.6	19.0	27.9
Total EtOH reacted	26.6	28.6	48.3	28.9	51.9	29.9	40.2	51.8	31.8	41.1	50.8

DISCUSSION OF RESULTS

The results of the experiments are summarized in Table I. In Fig. 1 is given a plot of the activity of the catalysts in the production of diethylaniline for gels differing in residual water. Included for comparison are curves from previous work on the decomposition of formic acid to carbon monoxide and water, using gels prepared from aluminum nitrate and chloride (9, Fig. 3). The points marked × on the second curve are per cent ethanol giving ether at

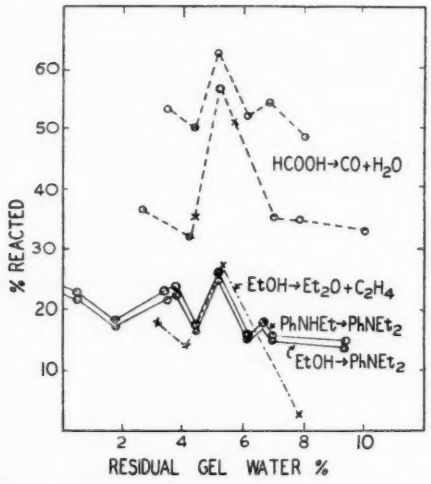

FIG. 1. The activity of catalysts differing in gel water.

250°C. (10). The bottom broken curve is for the production of both ether and ethylene at 318° (1).

It is apparent that alumina catalysts show a maximum in activity at 5.2% gel water. For the production of ethylene in the present work there is another maximum occurring between 2 and 4% gel water. This corresponds to the second maximum in the formic acid curve (9) and agrees with the position of the maximum in the ethylene to ether ratio (1, Fig. 1) although the latter differs from the point of maximum production of ethylene in the previous case.

That the activity of a catalyst may change with alteration in crystal lattice has been demonstrated by several workers (4, 8, 13). Taylor (14) and others (2, 6, 11) regard the catalytically active state of alumina as being due to the presence of gamma alumina having a cubic lattice A_0 7.90 (16), and consequently favor a method of preparation which is supposed to lead to the formation of this phase. Brown and Reid (3) however observed wide variations in the activity of their catalysts with slight variation in the method of preparation. Others have found no γ alumina in their most active catalyst (15).

The structural relations of the catalysts used in this work are shown in Figs. 2 and 3. In Fig. 2 X-ray patterns for catalysts M, N, I, and O, containing 7.0, 4.5, 3.5, and 1.8% residual water are obtained by plotting glancing angle as abscissa and intensity of the refracted beam as ordinate. No sharp peaks characteristic of crystalline samples were obtained. This indicates that the degree of crystallinity in all four samples was low, i.e. they were chiefly amorphous. Of these, catalysts M, N, and O exhibited low activity while catalyst I showed high activity. The patterns indicate no structural differences so that change in activity cannot be attributed to this factor.

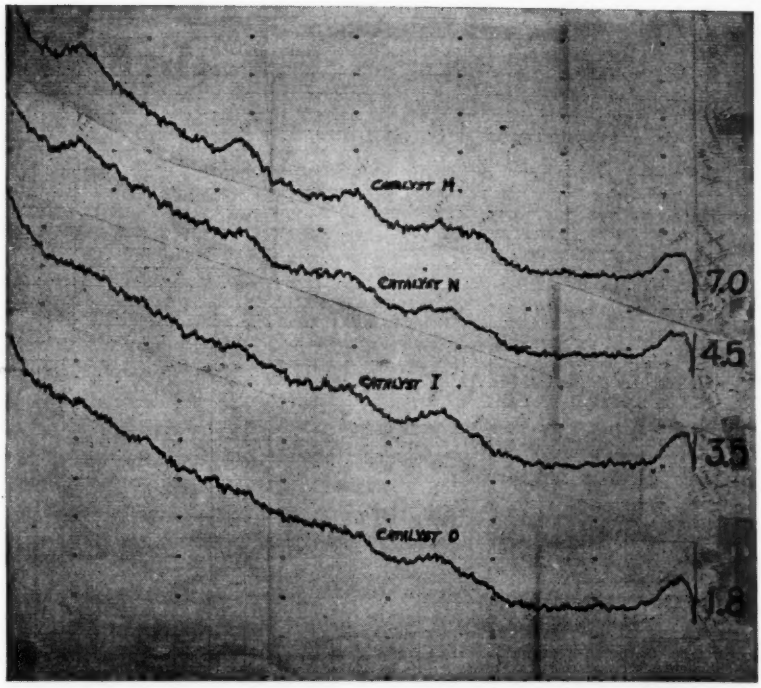

FIG. 2. X-ray diffraction patterns for catalysts containing 7.0, 4.5, 3.5, and 1.8% gel water. Catalyst I (3.5% water) had high activity, the others had low activity.

The X-ray photographs in Fig. 3 are for gels J, L, P, and Q, containing 3.8, 6.2, 5.2, and 0.0% gel water respectively. The first three obviously belong to the same type. The predominating structure appears to be that of Boehmite, with some gamma alumina. Catalysts J and P gave high activity, L much lower. These observations are similar to those made in the earlier work (9).

The catalyst containing zero water has a different structure. This catalyst also differs from that of zero water content used in the work cited above. The latter was prepared by blasting samples at 1200° resulting in conversion to alpha alumina or corundum. Such a catalyst showed a marked decrease in activity. In the present instance the anhydrous gel was obtained by activation in nitrogen at 550° which, according to Gregg and Sing (7), produces active gamma alumina. This is supported by its higher activity than the corundum sample and by the increase in activity over that of the gel containing 1.8% residual water.

Although an alumina catalyst containing 5.2% water had been found to be the best for the production of ether, both when ether alone is produced and when at higher temperatures ethylene and ether are formed, such a catalyst gave practically no ether in the present experiments. The very small amounts

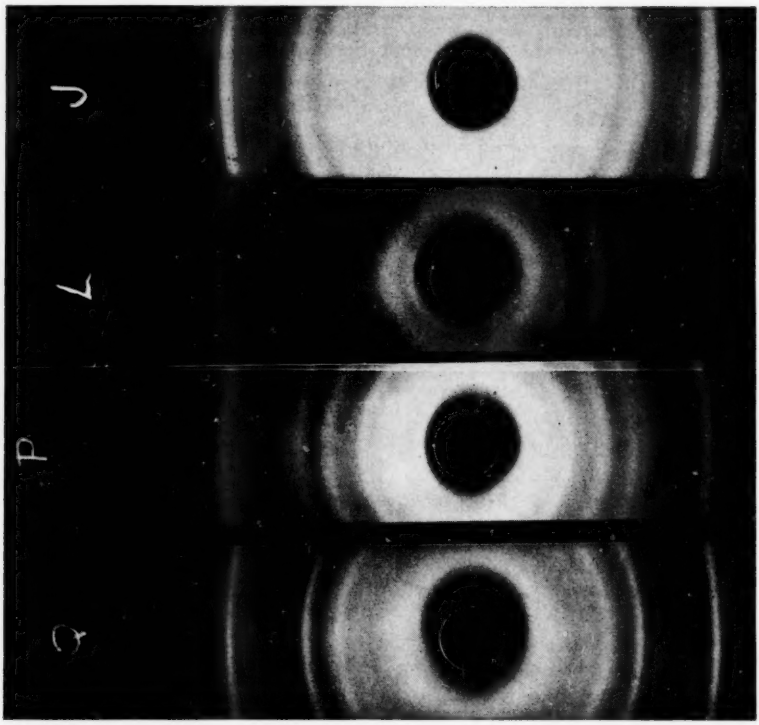

FIG. 3. X-ray photographs of catalysts containing 3.8, 6.2, 5.2, and 0% gel water.

of ether present in the reaction products can be explained if the reaction goes in two stages,

[1] $$2EtOH \rightarrow Et_2O + H_2O,$$

[2] $$Et_2O + PhNHEt \rightarrow PhNEt_2 + EtOH.$$

It was found that as the diethylaniline yield decreased the proportion of ethylene to diethylaniline decreased also. This would suggest that some of the ethylene was produced by decomposition of the reaction product, which might explain why maximum yields of ethylene were not obtained at the previously observed point on the activity – gel water curve.

SUMMARY AND CONCLUSION

From the above it may be concluded that while a change of surface geometry due to alteration of the crystal lattice can affect catalyst activity, the best and poorest surface condition of the catalyst may be established primarily by the extent to which constituent water is removed from the gel.

ACKNOWLEDGMENT

The authors wish to express their indebtedness to Dr. L. G. Berry, Department of Mineralogy, Queen's University, and to the management and staff of Aluminium Laboratories, Ltd., Arvida, for their assistance in obtaining X-ray diffraction patterns of the catalysts.

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CONDUCTANCES OF AQUEOUS LITHIUM NITRATE SOLUTIONS AT 25.0°C. AND 110.0°C.[1]

By A. N. Campbell, G. H. Debus[2], and E. M. Kartzmark

ABSTRACT

The conductances, densities, and viscosities of aqueous solutions of lithium nitrate have been determined, at temperatures of 25.0° and 110.0°C., at concentrations up to 14 molar. The results have been compared with the conductances calculated by means of the Robinson–Stokes equation and good agreement found up to about 7 molar. A short discussion of the theoretical implications is given.

In pursuance of our work on the conductances of concentrated solutions we have determined those of lithium nitrate solutions at 25° and 110°C. up to a concentration of 14 molar, which represents saturation at 110°C. We have also determined the viscosities and thus we have been able to compare our results with those calculated from the Robinson and Stokes equation (9). The agreement is surprisingly good.

After we had commenced this work, we came upon a paper by Klotschko and Grigorjew (7), in which the authors appeared to have anticipated our work completely, in that they have determined the specific conductances, viscosities, and densities of aqueous lithium nitrate solutions at concentrations varying from 0.22 to 71.64 weight per cent and at temperatures of 25°, 75°, and 100°. In this article and elsewhere (8), the authors prefer the specific to the equivalent conductance as a basis of theoretical interpretation. They also seek to use the equilibrium diagram for the system lithium nitrate – water to interpret their results. For instance, they state (with approximate truth) that the composition at which the maximum specific conductance occurs is that of the eutectic at which ice and lithium nitrate trihydrate coexist. However, on plotting the experimental values of specific conductance obtained by Klotschko and Grigorjew against concentration, and comparing them with our own, we feel that they are too inaccurate to be of great value. For example, the specific conductance of their weakest solution (at 25°) would give a value of the equivalent conductance in excess of the limiting value, calculated as the sum of the ionic conductances.

EXPERIMENTAL

The lithium nitrate used was a Fisher "Certified Reagent". The principal impurity was described as "other alkalis 0.35%". The lithium nitrate was recrystallized from water and small samples ignited in platinum, immediately before weighing. The more dilute solutions were made up by direct weighing, while solutions which were supersaturated at room temperature were analyzed by the conductance of a weighed aliquot. All the details of conductance measurements have been previously described (3). Viscosity measurements

[1]*Manuscript received June 22, 1955.*
Contribution from the Department of Chemistry, University of Manitoba, Winnipeg, Manitoba.
[2]*Holder of an N.R.C. Postdoctorate Fellowship.*

at 25° were made in a viscometer designed by Cannon and Fenske (2), while at 110° the simple apparatus of Fig. 1 was used. This apparatus was sealed after it had been filled, to prevent boiling and evaporation of the liquid; it was calibrated with water. Obviously, the total volume of liquid in the viscometer is not critical, so long as there is sufficient to fill the calibrated bulb. A number of different viscometers were used, having different capillary bores, in order to get a suitable flow time; this was never less than 30 sec. and, since time was measured with a stop-watch to one-fifth second, the accuracy of our viscosity determinations is better than one per cent, as far as this factor is concerned. As previously described (5) our high temperature density measure-

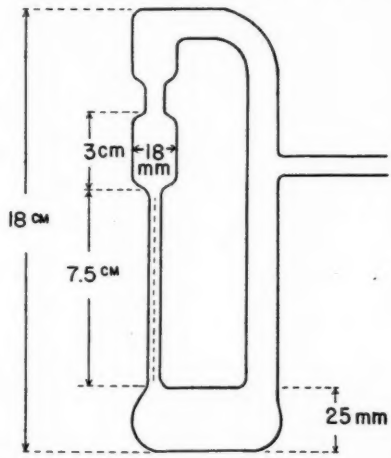

FIG. 1. Viscosity apparatus for high temperatures.

ments, carried out in a closed bomb, are probably not better than one in a thousand but this should not appreciably affect the viscosity. The apparatus (Fig. 1) could be rotated through 180°, while still in the thermostat, by means of a rack and pinion, and was automatically set in the same vertical position. The whole apparatus was placed in a tall thermostat filled with silicone. Temperature (at 110°) was maintained constant (using an Archimedean stirrer) within 0.1° which was sufficient for the purpose of our viscosity measurements. Temperature control for the conductance measurements was much better than this. For details of the high temperature conductance work, the paper by Campbell, Kartzmark, Bednas, and Herron (5) should be consulted.

EXPERIMENTAL RESULTS

The experimental results are contained in Table I, which is self-explanatory.

DISCUSSION OF RESULTS

As usual, the specific conductance is observed to pass through a maximum at about 5.337 molar at 25°, and 5.459 molar at 110°; the composition of the

TABLE I

CONDUCTANCES, DENSITIES, AND VISCOSITIES OF LITHIUM NITRATE SOLUTIONS

Wt. %	Conc., in moles per liter	Density, gm. per ml.	Relative viscosity, water = 1.0	Specific cond. κ, in mhos	Λ_{exp}	$\Lambda\eta$	Λ_{calc} $\mathring{a}$ = 4.5Å	Λ_{calc} $\mathring{a}$ = 5.0Å	Temperature coefficients: Conductance	Temperature coefficients: Viscosity
Temperature = 25°C.										
0.0635	0.00997	0.997	1.005	0.001025	102.79	103.31	102.3	102.5		
0.6878	0.0990	1.001	1.013	0.009083	91.74	92.93	90.88	91.46		
3.739	0.553	1.018	1.063	0.04244	76.86	81.70	75.18	76.82		
6.384	0.958	1.035	1.109	0.06730	70.26	77.89	68.75	70.15		
6.624	0.995	1.036	1.111	0.06942	69.78	77.50	68.33	69.86		
12.13	1.885	1.070	1.221	0.1112	58.95	71.98	58.22	59.44		
17.13	2.740	1.103	1.351	0.1387	50.60	68.35	50.41	52.01		
18.35	2.957	1.111	1.390	0.1448	48.98	68.06	—	—		
21.09	3.456	1.130	1.490	0.1545	44.72	66.65	44.44	45.96		
22.39	3.698	1.139	1.534	0.1583	42.81	65.70	42.78	44.26		
28.32	4.857	1.182	1.826	0.1685	34.68	63.31	34.89	35.86		
30.65	5.337	1.201	1.984	0.1694	31.66	62.81	31.43	32.58		
32.37	5.703	1.215	2.122	0.1685	29.54	62.67	—	—		
37.88	6.916	1.259	1.637	0.1626	23.52	62.06	22.69	23.50		
40.09	7.427	1.277	1.914	0.1578	21.24	61.89	20.31	20.99		
45.46	8.726	1.323	3.849	0.1441	16.51	63.54	14.86	15.40		
46.97	9.124	1.339	4.218	0.1388	15.21	64.15	13.34	13.93		
47.02	9.135	1.340	4.236	0.1387	15.18	64.30	—	—		
50.33	9.986	1.368	5.142	0.1277	12.79	65.76	10.81	11.18		
55.56	11.50	1.427	7.987	0.1067	9.28	74.03	6.67	7.15		
57.66	12.17	1.456	—	0.0972	7.99	—	—	—		
62.36	13.55	1.498	14.88	0.0817	6.03	89.72	3.45	3.56		
Temperature = 110°C.							Λ_0=360 $\mathring{a}$=3.0Å	Λ_0=356 $\mathring{a}$=3.5Å		
0.701	0.0972	0.956	1.010	0.02765	284.56	287.4	285.39	284.53	2.336	2.950
3.499	0.4936	0.973	1.053	0.1114	225.71	237.7	222.55	225.86	2.223	2.953
6.41	0.9177	0.988	1.094	0.1843	200.81	219.7	194.30	199.57	2.166	2.987
12.14	1.802	1.024	1.185	0.2955	164.03	194.4	165.97	166.22	2.045	3.020
17.25	2.634	1.053	1.276	0.3649	138.53	176.8	137.26	144.87	1.971	3.127
20.49	3.198	1.076	1.346	0.3989	124.75	167.9	125.73	133.22	1.960	3.218
26.25	4.252	1.117	1.516	0.4390	103.25	156.5	104.33	111.51	1.958	3.364
32.45	5.459	1.160	1.739	0.4476	82.00	142.6	85.78	92.01	1.961	3.710
40.13	7.109	1.222	2.197	0.4408	62.00	136.2	63.65	68.50	1.974	3.956
45.67	8.411	1.270	2.658	0.4254	50.57	134.4	50.20	54.20	2.187	4.427
49.00	9.237	1.300	3.097	0.4127	44.67	138.3	41.88	45.35	2.351	4.585
55.90	11.06	1.364	4.481	0.3705	33.50	150.1	27.29	29.24	2.687	
60.40	12.31	1.405	5.48	0.3658	29.72	162.8	17.82	23.37	3.31	
67.10	14.36	1.476	7.92	0.3317	23.10	182.9	13.76	15.24	4.22	

maximum changes but little with temperature. The very low value (6.03 mhos) to which the equivalent conductance falls, at 25°, is remarkable. It should be noted that the last four figures at 25° represent supersaturated solutions, which are easy to obtain. Although no particular interest now attaches to the calculation, the values of $\Lambda\eta$ have been worked out and a glance at column 7 of Table I shows that, as usual, the product drops rapidly from 103 at 0.01 molar to 68 at 2.74 molar, after which it drops very slowly to 61.9 at 7.4 molar, and then rises slowly to 89.7 at 13.6 molar (all at 25°). Similar behavior is observed at 110°. If the Robinson–Stokes equation were valid throughout the concentration range, it is easy to see from the form of the equation that the product should decrease continuously. The concentration

at which the product actually starts to increase (about 7 M) is the concentration beyond which the Robinson–Stokes equation is no longer valid.

In order to calculate Λ by means of the Robinson–Stokes equation it is necessary to know Λ_0 and to know (or assume) å, the distance of closest approach. In order to obtain the (theoretical) constants (B, B_1, and B_2) of the Robinson–Stokes equation, it is also necessary to know the dielectric constant and viscosity of water. The dielectric constant of water is now known up to the critical temperature of water (1), while good values for the viscosity of water are available up to 125° (6). Hence, at 25°, the only doubtful parameter is the distance of closest approach of the ions. We have made two sets of calculations, using å = 4.5 Å and å = 5.0 Å. It will be observed that both values give rather good agreement up to 7 M, but å = 4.5 Å is the better value, on the whole. Observed and calculated results are graphed in Figs. 2 and 3. At 110° we had the usual difficulty of not knowing Λ_0. We have assumed

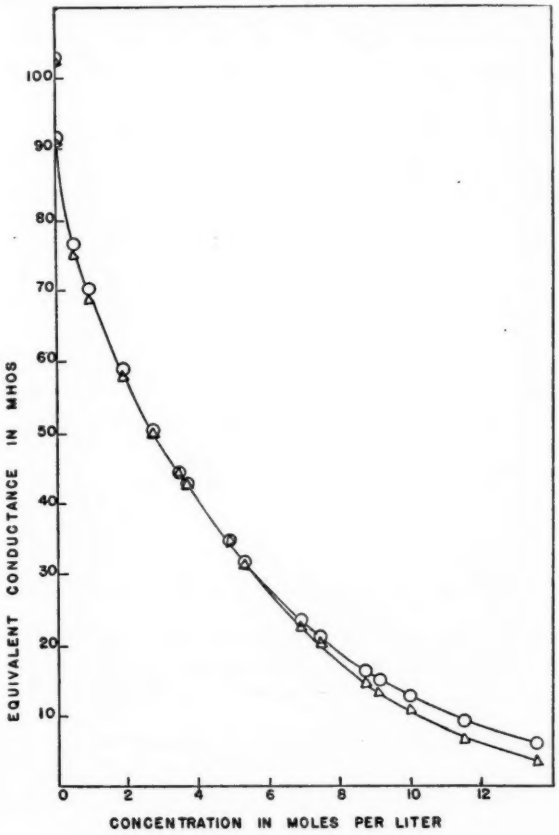

FIG. 2. ○ Experimental at 25°.
△ Calculated Λ_0 = 110.13, å = 4.5.

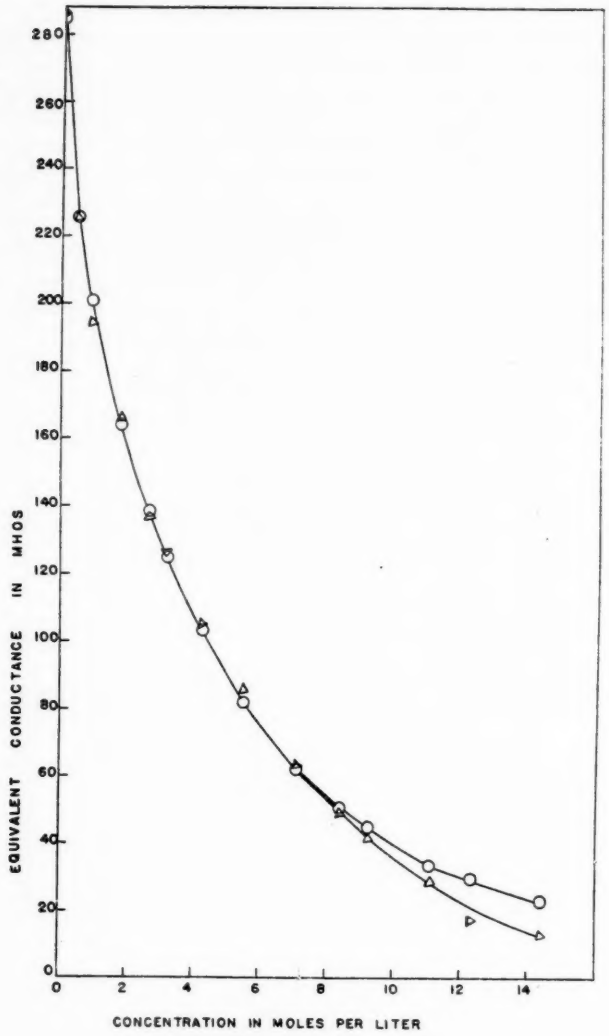

FIG. 3. ○ Experimental at 110°.
△ Calculated $\Lambda_0 = 360$, $\mathring{a} = 3.0$.

a value of $\Lambda_0 = 360$ mhos when using $\mathring{a} = 3.0$ Å, and of $\Lambda_0 = 356$ mhos when using $\mathring{a} = 3.5$ Å. Using the former set of values we obtain reasonably good agreement up to 4.2 *M*. Considering the uncertainty of Λ_0, better results can hardly be expected.

Despite the insistence of Stokes himself that å must not be a temperature function, we have boldly used a much smaller value for å at 110° than that

at 25° and for this we consider there may be justification. It is widely admitted that the lithium ion is highly and strongly hydrated. It seems reasonable to suppose, however, that the coordination number of hydration will decrease at higher temperatures and hence the distance of closest approach become smaller. Alternatively, if the coordination number does not change, or does not change very much, it may be that the attached water molecules may become more mobile at the higher temperature, and that they can then be pushed aside and thus allow the electrical centers of the ions to come closer together.

The agreement between calculated and observed equivalent conductances for lithium nitrate is so good, that it has not been necessary to resort to the hypothesis of ion-pair formation, though such an explanation has been offered to explain the fact that the calculated equivalent conductances are higher than the experimental values for silver nitrate and for ammonium nitrate. On the other hand, the agreement is good for ammonium chloride and potassium chloride (4). It seems reasonable to attribute ion-pair formation to the unsymmetrical form of the nitrate ion and therefore the Robinson–Stokes equation should be tested by further work on chlorides, etc.

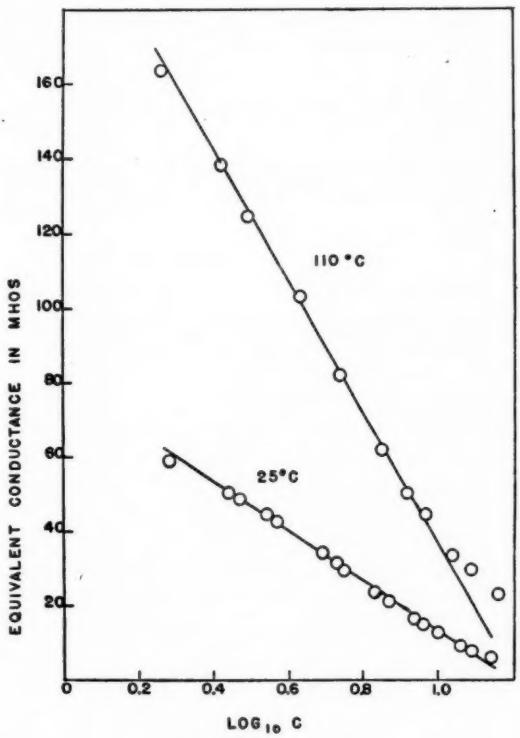

FIG. 4. Λ vs. log C.

We have elsewhere alluded to the fact that if equivalent conductance is plotted against the logarithm of the concentration, a straight line is obtained in the region of high concentration (5). The same effect is observed with lithium nitrate (Fig. 4), the straight line behavior beginning at about the concentration (say 5 to 7 molar) at which the Robinson–Stokes equation begins to break down. In the case of lithium nitrate, however, the straight line relation also breaks down at the very highest concentrations, where the lithium ion must be losing its water of hydration. If a coordination number of four is assigned to the lithium ion (Stokes believes it to be much higher than this) there must, at high concentrations, be insufficient water present to hydrate the ion. The lithium nitrate concentration corresponding to $Li^+.4H_2O$ is 49%; we have proceeded to a lithium nitrate concentration of 67% (by weight), which corresponds to less than $2H_2O$.

Finally, in the last two columns of the table of numerical results (Table I) the temperature coefficients of conductance and of viscosity have been obtained, assuming that both are straight line functions of temperature (they are not) between 25° and 110°. As usual, they are not equal and they behave in a somewhat erratic manner. The very high values of the temperature coefficient of viscosity in the region of high concentration (as high as 4.585 per degree) are, however, significant. These high values are in harmony with our belief that the lithium ion loses water of hydration at the higher temperature. This may also be true of the silver and ammonium ions but we see no reason to believe that these ions have any chemical or firmly bound water molecules. No doubt, all ions bind large numbers of water molecules in a more or less loose fashion, analogous to adsorption, but water of this kind will scarcely affect the distance of closest approach, or the viscosity, very much.

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STEROIDS AND RELATED PRODUCTS. III. THE SYNTHESIS OF 17β-METHYL-17-ISODESOXYCORTICOSTERONE[1,2]

By Ch. R. Engel[3] and G. Just[4]

ABSTRACT

The synthesis of 17β-methyl-17-isodesoxycorticosterone, an epimer of the biologically active hormone homologue 17α-methyldesoxycorticosterone, is described.

In the first communication of this series (5) and in a second paper (7) syntheses of 17α-methyldesoxycorticosterone (IV), a biologically active homologue of the adrenal cortical hormone desoxycorticosterone (I), were reported. The present communication deals with the synthesis of 17β-methyl-17-isodesoxycorticosterone (XII), a new homologue of the "unnatural" 17-isodesoxycorticosterone (14).

The synthesis proceeded from methyl Δ^4-3-keto-17β-methyl-17-isoetienate (VI); this substance has previously been obtained (5) together with its 17 epimer V by a rearrangement of the Aston–Greenburg or Faworsky type (2, 6) of 21-chloroprogesterone (II*b*) or of a mixture of this compound with 21-tosyloxyprogesterone (II*a*), as well as by Oppenauer oxidation of methyl Δ^5-3β-hydroxy-17β-methyl-17-isoetienate (III), an intermediate in the synthesis of 17-β-methyl-17-isoprogesterone (8). The transformation of the not easily saponifiable keto ester VI to the keto acid VIII was carried out similarly to the experiments in the natural series (5). The alkaline sensitive α,β-unsaturated ketone was protected by the formation of the enol ethyl ether IX, the latter was heated at high temperatures in a sealed tube with methanolic potassium hydroxide, and, after acidification of the reaction product, the free acid VIII isolated in good yield.

We decided to transform the acid VIII to the diazoketone X through the acid chloride VII, to convert the diazoketone X to the chloroketone XI, and to substitute the highly inert chlorine atom of the latter by an acetoxy grouping (XII*a*), using the method described previously (5, 7). As reported earlier, the direct hydrolysis of the 17 epimer of the diazoketone X to the epimer of the ketol XII had been possible under well-defined conditions, but the ketol obtained was accompanied by appreciable amounts of the corresponding chloroketone (5).

In the series of experiments described in this paper the acid chloride VII was at first prepared from the sodium salt VIII*a* by the action of oxalyl chloride in the presence of pyridine, according to a method originated by Adams and Ulich (1) and developed in the field of steroid chemistry by Wilds and

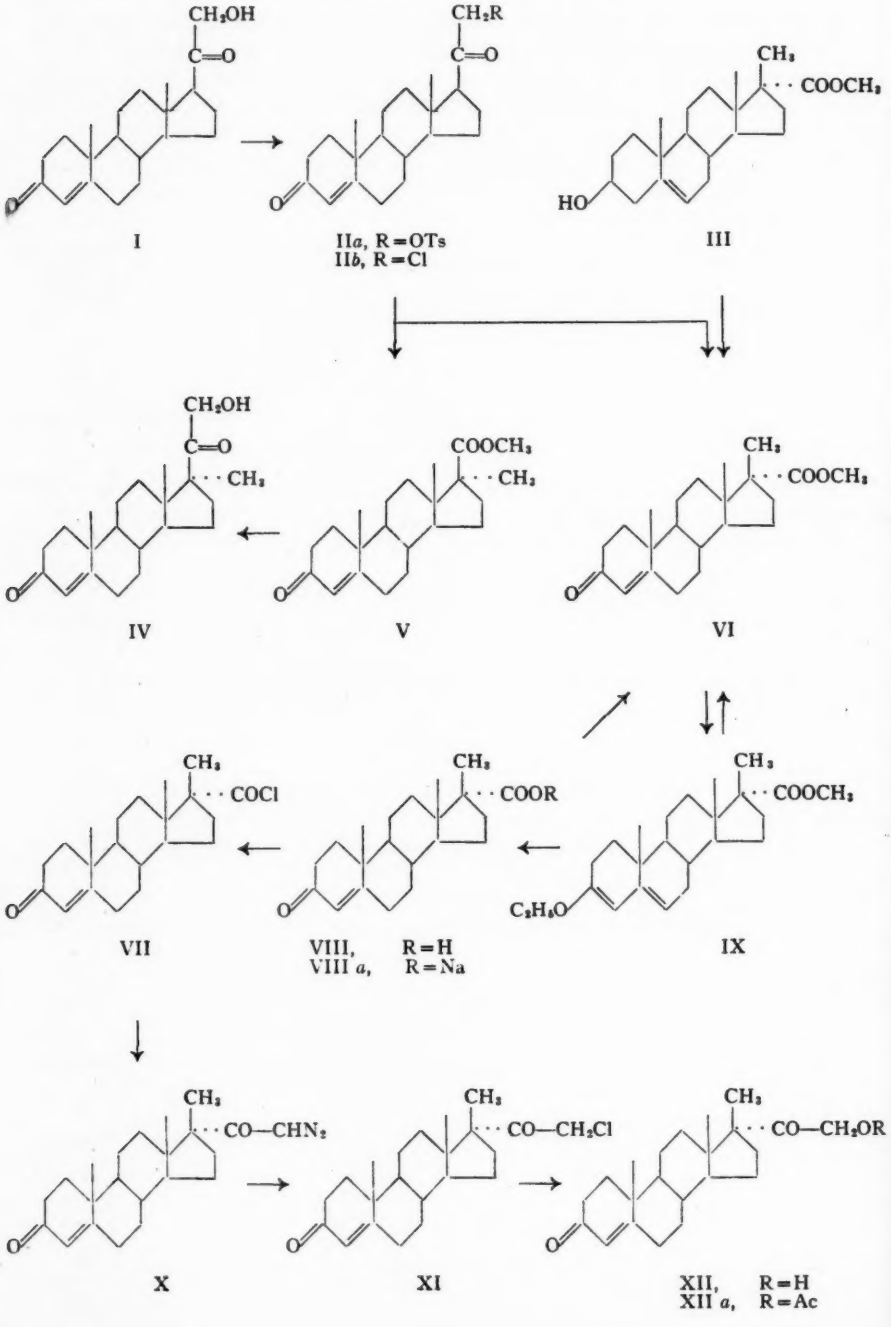
[1]*Manuscript received May 9, 1955.*

Contribution from the Collip Medical Research Laboratory and the Department of Chemistry, the University of Western Ontario, London, Ontario, Canada. Abstracted from part of the Ph.D. thesis of G. Just, to be presented to the Faculty of Graduate Studies of the University of Western Ontario. The subject of this publication was part of a communication presented before the annual meeting of the Chemical Institute of Canada, Toronto, June, 1954.

[2]*For Paper II of this series see Reference 4.*

[3]*Holder of a Medical Fellowship of the Canadian Life Insurance Officers Association.*

[4]*Holder of an Ontario Research Council Special Fellowship 1953–54 and of a National Research Council Studentship 1954–55.*



I

IIa, R = OTs
IIb, R = Cl

III

IV

V

VI

VII

VIII, R = H
VIII a, R = Na

IX

X

XI

XII, R = H
XII a, R = Ac

Shunk (16, 17). The crude acid chloride prepared by this procedure was subjected to the action of diazomethane, under the experimental conditions employed in the 17 α-methyl series (5), and the resulting crude diazoketone X was treated with an excess of hydrogen chloride in ether–benzene (compare 7, 12, 15). The desired chloroketone XI was thus obtained but the yields in this series of reactions were unsatisfactory. Reichstein and co-workers (13) have lately experienced similar difficulties in obtaining in good yield Δ^4-3-keto-21-diazopregnene from Δ^4-3-ketoetienic acid, using Wild's experimental conditions (16, 17) for the preparation of the intermediate acid chloride. However, the Swiss authors obtained satisfactory results when subjecting the free acid to the action of oxalyl chloride without addition of pyridine, a procedure which had also been developed as a general method by Adams and Ulich (1). In a second series of experiments we therefore applied Reichstein's experimental conditions (10, 13); the crude acid thus obtained was then transformed to the chloroketone XI, as mentioned above. The yields in this series of reactions showed a marked increase over those obtained when using the acid chloride prepared from the sodium salt VIII*a*. The very inert chlorine atom of the chloroketone XI was replaced by an acetoxy grouping by the action of silver acetate in acetic anhydride – pyridine (compare 5, 7). The resulting 17β-methyl-17-isodesoxycorticosterone acetate (XII*a*) was hydrolyzed to the free 17β-methyl-17-isodesoxycorticosterone (XII) by the prolonged action of potassium bicarbonate in methanol–water at room temperature and in a nitrogen atmosphere. Acetic anhydride in pyridine reacetylated the free ketol XII to the acetate XII*a*.

Similar to the findings in the 17β-methyl-17-isoprogesterone series (8) and in the 17-isoprogesterone (3) and 17-isodesoxycorticosterone series (14), all the here-described 17β-methyl-17-isosteroids are more levorotatory than their stereoisomers with the "natural" configuration in position 17.

Using a modification of the bioassay described by Marcus *et al.* (11), 17β-methyl-17-isodesoxycorticosterone acetate was found to produce no sodium retention in adrenalectomized fasting rats, at dose levels up to 300 μgm. Desoxycorticosterone acetate showed marked activity in the same test at 6.25 μgm.[5] This lack of biological activity affecting the electrolyte metabolism is in contrast to the life maintaining action of 17α-methyldesoxycorticosterone acetate (7), and shows close analogy to the inactivity of 17-isodesoxycorticosterone acetate in the life maintenace test (14) and to the lack of progestational activity of 17β-methyl-17-isoprogesterone (8) and of 17-isoprogesterone (3).

EXPERIMENTAL[6,7,8]

Methyl $\Delta^{3,5}$-3-Ethoxy-17β-methyl-17-isoetiadienate (IX)

Following the procedure previously described by Julian and co-workers (9)

[5] *The biological tests were kindly carried out by Professor J. A. F. Stevenson, Department of Physiology, University of Western Ontario. Details of these experiments will be published elsewhere.*

[6] *All melting points were taken in evacuated capillaries and the temperatures corrected.*

[7] *The microanalyses were carried out by Mr. J. F. Alicino, Metuchen, N.J., to whom we wish to express our high appreciation.*

[8] *The aluminum oxide used in the chromatographic absorption was treated as described under footnote 31 of Reference (5). We wish to thank Messrs. Merck and Co., Montreal, for kindly providing us with their aluminum oxide for chromatography.*

and by Engel and Just (5), 1.633 gm. of ester VI, m.p. 152–154°, was transformed to its enol ethyl ether. The crude product afforded upon recrystallization from acetone–methanol, in the presence of a few drops of pyridine, 1.48 gm. of IX, m.p. 105–106°, and a second crop of 155 mgm., m.p. 100–106° (92.6%). A sample was recrystallized three times for analysis; shiny plates, m.p. 109–110°, $[\alpha]_D^{21}$ −132.8° (*c*, 0.982 in $CHCl_3$–pyridine). Anal. Calc. for $C_{24}H_{36}O_3$: C, 77.37; H, 9.74. Found: C, 77.20; H, 9.72.

Hydrolysis.—Following the experimental conditions described previously (5), 53 mgm. of enol ether IX was hydrolyzed with hydrochloric acid in methanol–water. Crystallization of the amorphous reaction product from acetone–hexane afforded 35 mgm. of the ester VI, m.p. 151–152.5° (71%). Further crystallization raised the melting point to 153–154°. The melting point was not depressed upon admixture of authentic ester VI.

The mother liquors from the recrystallizations of ether IX were subjected to a similar hydrolysis. Purification of the crude reaction product by chromatography and crystallization gave 33 mgm. of ester VI, m.p. 147–152°. Thus the total yield of ether IX from keto ester VI was raised to 94.5%.

Δ^4-3-Keto-17β-methyl-17-isoetienic Acid (VIII) from Enol Ether IX

In a sealed tube 1.636 gm. of enol ether IX was heated with 80 cc. of a 7% methanolic potassium hydroxide solution for 48 hr. at 168–170°. After cooling, the reaction mixture was poured into water and acidified with sulphuric acid. The chloroform extraction of the precipitate gave 1.663 gm. of crude acid, m.p. 203–207°, which afforded upon recrystallization from acetone 982 mgm. of long blades, m.p. 217–222° (62.6%). A sample was recrystallized four times for analysis; m.p. 223–225°, $[\alpha]_D^{24}$ 83.3° (*c*, 1.286 in $CHCl_3$). Anal. Calc. for $C_{21}H_{30}O_3$: C, 76.33; H, 9.15. Found: C, 76.60; H, 9.26.

Methylation of acid VIII.—Acid VIII (110 mgm.) was dissolved in absolute ether and methylated with an ethereal solution of diazomethane at 0° for 15 hr. The solvent was removed *in vacuo*. Chromatography of the resulting amorphous product (150 mgm.) on 5 gm. of aluminum oxide and recrystallization of the petroleum ether – benzene fractions from acetone–hexane yielded 79 mgm. of colorless needles, m.p. 150–152°, which gave no depression of melting point upon admixture of authentic ester VI.

Similar methylation of the mother liquors from the recrystallizations of acid VIII (545 mgm.) afforded 262 mgm. of ester VI, m.p. 144–152°. Considering this recovery of starting material, the yield of the saponification was 74.5%.

21-Chloro-17β-methyl-17-isoprogesterone (XI)

(*a*) *From the sodium salt of Δ^4-3-keto-17β-methyl-17-isoetienic acid (VIIIa).*—According to the procedure described previously (5, 16, 17), 1 gm. of acid VIII was transformed to its sodium salt, which after being dried for 10 hr. at 105° in a high vacuum was suspended in 21 cc. of absolute benzene containing seven drops of pyridine and treated with 10 cc. of oxalyl chloride at −15°; after 25 min. the temperature was raised to 25°, and after a further 15 min. the mixture was worked up in the usual manner. A solution of the product in 25 cc. of absolute benzene was slowly added, with vigorous stirring, to 33 cc.

of a 3.3% ethereal diazomethane solution at −15°. The mixture was kept seven hours at −5° and 90 min. at room temperature, and the excess solvents were removed *in vacuo*. To a suspension of this crude diazoketone in 50 cc. of absolute ether was added, with swirling, a solution of 876 mgm. of hydrogen chloride in 20 cc. of absolute ether. After 45 min., the reaction mixture was poured into water; the ether extraction of the precipitate gave 1.5 gm. of an oil which was chromatographed on 30 gm. of aluminum oxide. Recrystallization of the petroleum ether – benzene (4:1; 1:1; 1:4) and benzene elutions from acetone–hexane afforded 341 mgm. of the chloroketone, m.p. 175–180° (31%). A sample was recrystallized from acetone–hexane five times for analysis; colorless plates, m.p. 182.5°, $[\alpha]_D^{24}$ 43.2° (*c*, 1.02 in $CHCl_3$), λ_{max}^{EtOH} 239 mμ (log ε 4.2). Anal. Calc. for $C_{22}H_{31}O_2Cl$: C, 72.80; H, 8.61, Cl, 9.77. Found: C, 72.97; H, 8.81; Cl, 9.73.

(*b*) *From acid VIII.*—According to Reichstein's modification (10, 13) of Wilds' method (16, 17), 3.3 cc. of oxalyl chloride in 8.2 cc. of absolute benzene was added to a suspension of 660 mgm. of Δ^4-3-keto-17β-methyl-17-isoetienic acid (VIII), m.p. 221–222°, in 25 cc. of benzene at 0°. The acid went into solution after five minutes. The mixture was shaken for 45 min. at frequent intervals at room temperature. The excess solvents were removed *in vacuo* at 15° and the reaction product was dried with absolute benzene at 20°. The solution of the crude acid chloride VII in 18 cc. of absolute benzene was added slowly, with stirring and exclusion of moisture, to 26 cc. of a 1.8% ethereal diazomethane solution at −15°. The mixture was allowed to stand at 0° for 18 hr., then 30 min. at room temperature. The solvents were removed *in vacuo* at 15°. The crude diazoketone X was treated with 402 mgm. of hydrogen chloride in ether–benzene, as described above. The amorphous reaction product (950 mgm.) gave upon chromatography and recrystallizations 16 mgm. of methyl ester VI, m.p. 146–151°; 235 mgm. of 21-chloro-17β-methyl-17-isoprogesterone (XI), m.p. 178–181°; 83 mgm. of XI melting at 173–177° (43.9%); and 60 mgm. crude acid VIII. Methylation of the latter gave after chromatographic purification 40 mgm. of ester VI, m.p. 147–153°. Taking into account this recovery of acid, the yield of the chloride was 47.5%.

17β-Methyl-17-isodesoxycorticosterone Acetate (XIIa) from Chloroketone XI

To a solution of 560 mgm. of the chloroketone XI, m.p. 173–177°, in 7.7 cc. of acetic anhydride was added 1.8 gm. of silver acetate in 6.4 cc. of hot pyridine. The mixture was refluxed for 100 min. at 135–140° bath temperature in a nitrogen atmosphere. The usual working up gave 600 mgm. of a dark brown oil which was dissolved in 8 cc. of benzene and chromatographed on 20 gm. of aluminum oxide. Petroleum ether – benzene (1:4), benzene, and benzene–ether (95:5; 4:1) eluted 115 mgm. of fine needles, m.p. 140–154° (19.2%). A sample was recrystallized three times for analysis; m.p. 154.5–155.5°, $[\alpha]_D^{26}$ 33.2° (*c*, 1.11 in $CHCl_3$), λ_{max}^{EtOH} 241 mμ (log ε 4.24). Anal. Calc. for $C_{24}H_{34}O_4$: C, 74.57; H, 8.87. Found: C, 74.62; H, 8.68.

17β-Methyl-17-isodesoxycorticosterone (XII)

To a solution of 83 mgm. of the acetate XII*a*, m.p. 150–153°, in 7.6 cc. of methanol was added 83 mgm. of potassium bicarbonate in 1.4 cc. of water.

The mixture was kept at room temperature in an atmosphere of nitrogen for four and one-half days. The usual working up afforded 72 mgm. (97.3%) of needles, m.p. 134–136°, giving a depression of the melting point upon admixture of the acetate XII*a*. The product was recrystallized three times for analysis; m.p. 137.5–138°, $[\alpha]_D^{22}$ 60.5° (*c*, 0.82 in $CHCl_3$), λ_{max}^{EtOH} 241 mμ ($\log\epsilon$ 4.23). Anal. Calc. for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.65; H, 9.23.

Acetylation.—Isodesoxycorticosterone XII (66 mgm.) was acetylated with 1 cc. of acetic anhydride in 2 cc. of pyridine and worked up in the usual way. The resulting product (79 mgm.) crystallized upon trituration with ether–hexane, m.p. 131–145°. The product was chromatographed on 3 gm. of aluminum oxide. Recrystallization of the petroleum ether – benzene (1:4), benzene, and benzene–ether (4:1) elutions from ether–hexane afforded 25 mgm. of the acetate XII*a*, m.p. 151–153°, and 20 mgm. melting at 145–148°. Admixture of authentic acetate XII*a* gave no depression of the melting point.

ACKNOWLEDGMENTS

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CARBOHYDRATES OF SUNFLOWER HEADS[1]

By C. T. Bishop

ABSTRACT

Extractive-free, ash-free sunflower seed heads contained lignin (12.3%), pectin (27.5%), alkali-soluble polysaccharides (8.2%), and α-cellulose (52.0%). Pectin and polysaccharides were separated by successive extraction of the seed heads with ammonium oxalate – oxalic acid solution and 5% aqueous sodium hydroxide. The pectin was 37% esterified with methyl groups and yielded only galacturonic acid when hydrolyzed. The polysaccharides yielded, on hydrolysis, a mixture of neutral sugars and an aldobiouronic acid. The neutral sugars consisted of D-xylose (59.3%), D-glucose (23.5%), D-galactose (16.2%), and traces of arabinose and rhamnose. Fractionation of the polysaccharide acetate indicated that the three main sugars were constituents of three different polysaccharides. The aldobiouronic acid (1.53% of the polysaccharides) was shown to be 3-*O*-α-(D-glucopyruronosyl)-D-xylopyranose.

INTRODUCTION

Sunflowers are grown commercially in Southern Manitoba for the edible oils obtained from the seeds. The sunflower heads are not recovered after harvesting the seeds but are plowed back into the the ground and represent an agricultural waste of considerable importance. In 1948 Stoikoff (12) reported that sunflower seed heads gave good yields of pectin and suggested that they might become a commercial source for this product. Shewfelt (11) evaluated this possibility by studying the effect of isolation procedures on gelling properties of sunflower pectin. Highest yields of good-gelling preparations were obtained by extracting the sunflower seed heads with a hot dilute solution of ammonium oxalate – oxalic acid.

Pectin and polysaccharides are closely associated in plants and it has not been shown whether this association is chemical or physical (9). It has been difficult to isolate either of the two materials without concurrent degradation of the other. Polysaccharides are usually extracted by alkaline solutions which decompose pectin, and pectin is extracted by acidic solution which may hydrolyze the more labile polysaccharides (8).

This report deals with the effectiveness of the ammonium oxalate – oxalic acid extraction, developed by Shewfelt (11), for separating pectin from polysaccharides, and with the chemical composition and properties of these two products from sunflower seed heads.

Extraction Procedure

Diagram 1 shows the fractionation procedure used to separate the carbohydrate components of sunflower seed heads. Chromatography of Fraction 1 (mother liquors from precipitation of pectin) before and after hydrolysis showed the fraction to be free of sugars and proved that no degradation of the polysaccharides occurred during removal of the pectin. In a similar way, a hydrolyzate of Fraction 2 (pectin) was found to contain no sugars other than

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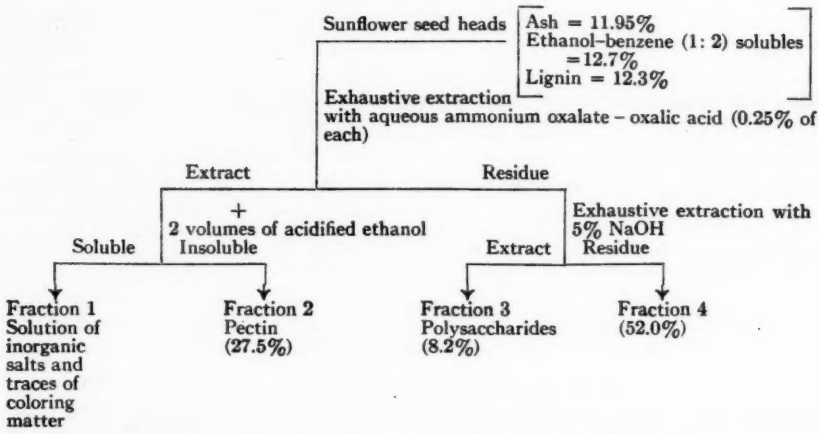
DIAGRAM 1

FRACTIONATION OF SUNFLOWER SEED HEADS*



Lignin analysis and yields of fractions are on an ash-free, extractive-free basis.

galacturonic acid, and therefore no polysaccharides were coextracted with the pectin. Complete removal of pectin by extraction with the ammonium oxalate – oxalic acid solution was confirmed by the absence of galacturonic acid in hydrolyzates of Fractions 3 (polysaccharides) and 4 (largely cellulose). These results showed that the pectin and polysaccharides of sunflower seed heads could be completely separated by first removing the pectin with a dilute solution of ammonium oxalate – oxalic acid. This procedure may prove useful in investigating carbohydrates of other plant materials when pectin interferes with the purification of polysaccharides.

Pectin

Sunflower pectin yielded only galacturonic acid on hydrolysis and was therefore devoid of contaminating polysaccharides. Ninety-seven per cent of the product was soluble in water, forming a clear 0.5% solution and having a specific rotation (+249°) in good agreement with that reported (+250°) by Luckett and Smith (10) for a highly purified sample of citrus pectin. Sunflower pectin, as isolated, contained 6% of methyl ester groups and was therefore only 37% esterified, fully esterified pectin requiring 16% of methyl ester groups. The pectin was fully esterified by diazomethane and then saponified, the difference in methoxyl contents corresponding to a uronic acid content of 95.6%. Oxidation of the pectin with nitric acid gave a 60% yield of mucic acid. Sunflower pectin was resistant to all attempts at methylation with (*a*) dimethyl sulphate and alkali, (*b*) diazomethane, (*c*) methyl iodide and silver oxide, and (*d*) thallous hydroxide and methyl iodide; hence no detailed structure could be determined. However, the properties and reactions described above were the same as those reported (8) for pectin from many other sources

and there seems little doubt that sunflower pectin has the same basic structure, i.e. D-galacturonic acid units joined by α, $1 \rightarrow 4$ glycosidic bonds.

Polysaccharides

Acid hydrolysis of the polysaccharides yielded an aldobiouronic acid (1.53%) and five neutral sugars: D-xylose (59.3%), D-glucose (23.5%), D-galactose (16.2%), arabinose (trace), and rhamnose (trace). The neutral sugars crystallized and were identified by melting points and rotations except for arabinose and rhamnose which were present in such small amounts that they could only be identified chromatographically.

Attempts were made to fractionate the polysaccharides to obtain a chemically homogeneous portion for structural studies. The polysaccharides gave a soluble and insoluble fraction with Fehling's solution, both containing all five monosaccharides in the same proportions.

The polysaccharide preparation was acetylated and fractionated by precipitation from chloroform solution with petroleum ether. Table I represents a

TABLE I

FRACTIONATION OF THE ACETATES OF POLYSACCHARIDES FROM SUNFLOWER HEADS

Fraction	Volume (ml.) of pet. ether per 400 ml. chloroform	Yield (gm.)	Rotation $[\alpha]_D^{25}$ (c = 1% in $CHCl_3$)	Constituent sugars*			
				Xylose	Glucose	Galactose	Arabinose
1	160	1.65	−47.6	85	10	5	Trace
2	250	1.71	−32.6				
3	365	2.71	+37.3	70	20	10	Trace
4	440	0.70	+93.8				
5	640	2.84	+123.0	25	40	35	Nil

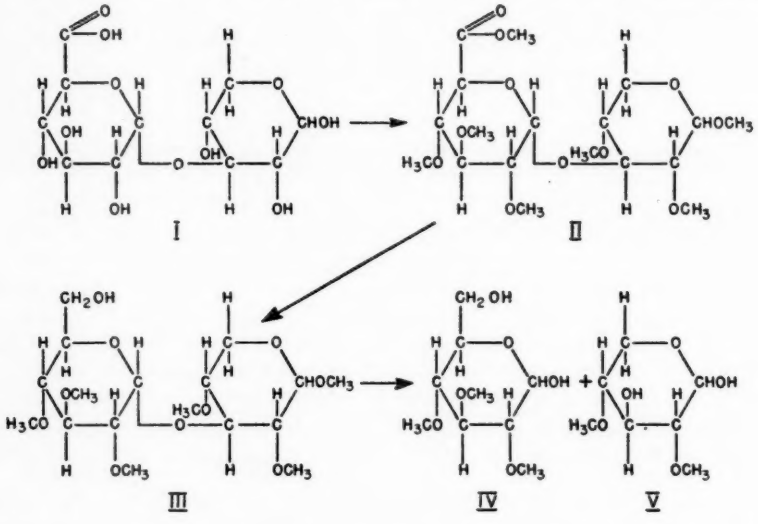
**No rhamnose could be found in any of the fractions. It may have been spread throughout all fractions in quantities too small for detection.*

fractionation of 10 gm. of this material. The differences in specific rotation indicated a mixture of chemically different polysaccharides and the change in a dextrorotatory direction corresponded with an increase in hexosan material and a decrease in pentosans. Naturally occurring xylans have high negative rotations, in the order of −90° to −110° after purification, and usually contain some arabinose units (13). It was probable, then, that the first two acetate fractions consisted of an arabo-xylan contaminated by some hexosan material. The results also showed that there was no constant ratio between any of the component monosaccharides, good evidence that the three main sugars were present in different polysaccharides: a levorotatory arabo-xylan, and a dextrorotatory glucosan and galactan. This is similar to results found by Gilles, Meredith, and Smith (7) who isolated three polysaccharides from barley gum: a levorotatory arabo-xylan and two polyglucosans, one dextrorotatory and the other levorotatory. However, the three polysaccharides of sunflower heads could not be completely separated either by repeating or by extending the fractionation, i.e. by further fractionation of each fraction. The difficulty in

separation was probably caused by similarities in molecular size of the components. Because of the dubiousness of interpreting results of methylation studies on a mixture of polysaccharides no further structural investigations were carried out.

Aldobiouronic Acid

The aldobiouronic acid (I), isolated from the hydrolyzate of the polysaccharides by ion exchange resins, had an acid equivalent of 318 (calculated 326) and yielded glucuronic acid and xylose when hydrolyzed. Its dextrorotatory specific rotation (+59.7°) indicated that the glycosidic linkage was in the α-configuration. This compound was methylated (II), reduced (III), and hydrolyzed to yield the partially methylated glucose (IV) and xylose (V) derivatives. The glucose fragment was identified as 2,3,4-tri-*O*-methyl-D-



glucopyranose (IV) by oxidation and esterification to the crystalline methyl ester of 2,3,4-tri-*O*-methyl-D-glucosaccharolactone. The xylose portion of the molecule was shown to be 2,4-di-*O*-methyl-D-xylopyranose (V) by conversion to the crystalline amide of 2,4-di-*O*-methyl-D-xylonic acid. These results proved that the aldobiouronic acid was 3-*O*-α-(D-glucopyruronosyl)-D-xylopyranose. This compound has also been isolated from xylans of pear cell wall (5) and wheat straw (1, 2).

EXPERIMENTAL

Chromatograms were run by the descending method using: (*A*) ethyl acetate – pyridine – water, 2:1:2; (*B*) ethyl acetate – acetic acid – water, 9:2:2; (*C*) butanol – acetic acid – water, 4:1:5; and (*D*) butanol – ethanol – water, 5:1:4. Sugars were detected on chromatograms by aniline oxalate or silver nitrate sprays. Evaporations were carried out under reduced pressure

at 40°C. or less. All melting points are corrected and rotations are equilibrium values.

Isolation of Pectin and Polysaccharides from Sunflower Heads

Sunflower seed heads (ash, 11.95%) were extracted exhaustively in a Soxhlet with ethanol–benzene, 1: 2 (v/v). This procedure removed 12.7% as waxes and pigments. All analyses and yields that follow were calculated on an ash-free, extractive-free basis. Extractive-free sunflower heads (Klason-lignin, 12.3%) were stirred in an aqueous solution of ammonium oxalate – oxalic acid (0.25% of each) at 75°C. for one and one-half hours. A solid: liquid ratio of 1: 22 was used. The extracts were removed by filtration through a linen cloth, and addition of two volumes of ethanol, acidified with 5 ml. of concentrated hydrochloric acid per liter, caused the formation of a gelatinous precipitate of pectin. The extraction procedure was repeated until no more ethanol-insoluble material was obtained, three extractions being required. The pH of the extracts varied from 3.6 to 4.0. The precipitated pectin (Fraction 2, Diagram 1) was filtered, with suction, on Whatman No. 1 paper, washed several times with ethanol and then with ether, and dried at 0.1 mm. over paraffin and phosphoric anhydride (yield, 27.5%). The alcoholic mother liquors (4 liters, Fraction 1, Diagram 1) from the pectin precipitation were evaporated to 250 ml. and examined by paper chromatography (solvent *A*). No sugars were detected. The solution was then made 2 *N* with respect to hydrochloric acid and was heated on a boiling water bath for six hours. The hydrolyzate was neutralized with Dowex-2 (carbonate form) and examined by chromatography as before. No sugars were found.

The pectin-free residue was stirred at room temperature in 5% aqueous sodium hydroxide using a solid: liquid ratio of 1: 20. After 24 hr. the extract was removed by suction filtration through linen cloth and the residue was extracted three more times in the same way. The combined extracts were neutralized with acetic acid and dialyzed in cellophane against distilled water for 36 hr. The solution of non-dialyzable material was evaporated to one-quarter its volume and diluted with three volumes of 95% ethanol to precipitate the polysaccharides. The precipitate was removed by centrifuging, washed several times with ether, and finally dried at 0.1 mm. over paraffin and phosphoric anhydride (yield 8.2%, Fraction 3, Diagram 1).

The residue from the alkali extraction of the polysaccharides was washed successively with 10% aqueous acetic acid, water, and acetone before drying *in vacuo* over phosphoric anhydride (yield 52.0%, Fraction 4, Diagram 1). This fraction was not examined further but probably consisted largely of cellulose.

Pectin

Ninety-seven per cent of sunflower pectin was soluble in water, forming a clear solution having $[\alpha]_D^{25} = +249°$ ($c = 0.49$). The pectin as isolated had a methoxyl content of 6.0% which was completely removed by saponification with alkali showing that the methoxyl groups were present as methyl esters. Since the calculated methoxyl content for fully esterified pectin ($C_6H_7O_6$.-

$OCH_3)_n$ is 16.3%, sunflower pectin was only 36.8% esterified. A sample (1 gm.) of pectin was allowed to stand at 5°C. for seven days in an ethereal solution of diazomethane. The diazomethane solution was replaced from time to time as the yellow color faded. Evaporation of the ether left fully esterified sunflower pectin as a white powder (1.07 gm., MeO = 19.5%). This fully esterified, partially etherified pectin was shaken with *N* alcoholic sodium hydroxide for 24 hr. to saponify the ester groups. The de-esterified pectin was recovered by centrifuging and after being washed with ethanol, ether, and petroleum ether, it was dried *in vacuo*. This product (0.98 gm.) was a white powder having a methoxyl content of 3.9%. The saponification therefore reduced the methoxyl content by 15.6% corresponding to a uronic acid content of 95.6%. Pectin (1 gm.) was heated on a steam bath with nitric acid (10 ml., sp. gr. 1.2) until the volume was reduced by about two-thirds, and the solution was kept at room temperature for 10 hr. Crystals of mucic acid (0.65 gm.) were filtered and recrystallized from alkaline solution by acidification with hydrochloric acid. The crystals had a melting point and mixed melting point with mucic acid of 212–213°C. A sample (50 mgm.) of sunflower pectin was heated in a sealed tube at 110°C. for 17 hr. with 2.5% sulphuric acid (10 ml.). The hydrolyzate was neutralized with barium carbonate and the neutral solution freed of barium by Amberlite IR-120. When this solution was examined by chromatography, using glucuronic acid, galacturonic acid, xylose, arabinose, and glucose as reference sugars, only galacturonic acid could be found with solvents *A*, *B*, and *C*.

Pectin (40 gm.) was methylated 10 times with dimethyl sulphate and potassium hydroxide to a constant methoxyl content of 11.9% (calculated for $C_8H_{11}O_6K$, MeO = 25.6%). This product (8.4 gm.) was then converted to the thallium complex, which was methylated with methyl iodide. This procedure gave a product (2.1 gm., MeO = 34.5%) which was methylated twice more with Purdie's reagents to a constant methoxyl content of 38.5% (calculated for $C_6H_5O_3{\cdot}3OCH_3$, MeO = 42.6%). Fractionation of this material from acetone by precipitation with petroleum ether did not yield any fractions with a higher methoxyl content. When sunflower pectin was allowed to stand under ethereal diazomethane at 5°C. for several weeks, the methoxyl content was raised to 29%. Further methylations of this product with Purdie's reagents, by the thallium – methyl iodide method, and with dimethyl sulphate and alkali gave low yields of products with methoxyl contents no higher than 37%.

Polysaccharides

The polysaccharides (Fraction 3, Diagram 1) of sunflower heads were isolated as a light, tan powder having $[\alpha]_D^{25} = +110°$ (c = 2% in 4% sodium hydroxide). Hydrolysis of a small sample (50 mgm.) by heating with 2% sulphuric acid (3 ml.) at 97° for 10 hr. released xylose, glucose, galactose, glucuronic acid, and traces of arabinose and rhamnose. These sugars were detected by paper chromatography in solvents *A*, *B*, *C*, and *D* using known sugars as reference compounds. The polysaccharides, treated with Fehling's

solution (4), gave an insoluble copper complex, which was separated by filtration. Polysaccharide material isolated from both the precipitate and filtrate, after removal of copper salts by acidification and dialysis, yielded the same sugars on hydrolysis as the original polysaccharide preparation. The polysaccharides (43.6 gm.) were acetylated twice by the formamide – pyridine – acetic anhydride method (3). The acetate (59.3 gm.) was fractionated from chloroform solution by successive precipitation with petroleum ether (60–100°C.). Table I represents the fractionation of 10 gm. of the acetate by this method. Constituent sugars were estimated, after deacetylation of small aliquots (*ca.* 10 mgm.) of each fraction by alcoholic potassium hydroxide, by hydrolysis and quantitative paper chromatography (6). This fractionation was carefully repeated five times on the total acetate mixture and was extended by further fractionation of the individual fractions. The procedure was also carried out in reverse by extracting the acetate mixture with petroleum ether containing increasing amounts of chloroform. No improvement of the separation shown in Table I could be obtained.

Identification of Component Sugars

Deacetylated polysaccharides of sunflower heads (30.27 gm.) were heated on a boiling water bath in 2% sulphuric acid (500 ml.). After 10 hr. an insoluble residue (2.11 gm.) was filtered and the filtrate was heated again to constant rotation, $[\alpha]_D^{25} = +27.6°$. The hydrolyzate was brought to pH 3.0 with saturated barium hydroxide solution to remove a large amount of the sulphuric acid and was clarified by centrifuging. To remove barium, the centrifugate was passed through a column of Amberlite IR-120, which was then washed with water until the eluates gave a negative anthrone test. The eluates were evaporated to 500 ml. and passed through a column of Amberlite IR-4B to remove uronic acids (see below) and last traces of sulphuric acid. Evaporation of this eluate left a mixture of neutral sugars (25.05 gm.) which was shown by quantitative paper chromatography (solvent *A*) to consist of xylose (59%), glucose (23%), and galactose (16%). Arabinose and rhamnose were also present in trace quantities. A portion (1 gm.) of this mixture of sugars was separated by preparative paper chromatography to yield: D-xylose, m.p. 146–147°C., $[\alpha]_D^{25} = +19.5$ ($c = 3.18$ in water); D-glucose, m.p. 143–144.5°C., $[\alpha]_D^{25} = +54.0$ ($c = 1.11$ in water); and D-galactose, m.p. 165–166°C., $[\alpha]_D^{25} = +78.0$ ($c = 0.41$ in water). Since the quantities of arabinose and rhamnose were insufficient for isolation, these two sugars could only be identified as being chromatographically indistinguishable from arabinose and rhamnose in solvents *A*, *B*, *C*, and *D*.

Aldobiouronic Acid

The uronic acids were removed from the column of Amberlite IR-4B by elution with *N* sulphuric acid until an anthrone test was negative. The eluate was neutralized (pH 7.0) with saturated, aqueous barium hydroxide and inorganic barium salts were removed by filtration. The filtrate, evaporated to 200 ml., was passed through a column of Amberlite IR-120 to remove barium from the uronic acids. Evaporation of the eluate and examination by

chromatography revealed some xylose still mixed with the uronic acids. This mixture (1.40 gm.) was separated on a cellulose column, the xylose being eluted first with water-saturated butanol. Butanol – formic acid, 50: 1, then removed traces of glucuronic acid, and this was followed by elution with water, which removed the aldobiouronic acid. Evaporation of the aqueous eluate left the aldobiouronic acid as a thick yellow sirup (429.2 mgm.) having $[\alpha]_D^{25} = +59.7$ ($c = 7.5$ in water) and an acid equivalent, by titration, of 318 (calculated for $C_{11}H_{18}O_{11}$, 326). The aldobiouronic acid (400 mgm.) in water (5 ml.) was added to dimethyl sulphate (5 ml.) and the mixture was stirred vigorously at 0°C. during the dropwise addition of 30% aqueous sodium hydroxide (7.5 ml.) over a period of eight hours. The mixture was then stirred for an additional 18 hr. during which it was allowed to warm to room temperature. The mixture was again cooled to 0°C. followed by the addition of solid sodium hydroxide (4.5 gm. all at once), and dimethyl sulphate (5 ml., drop by drop, during six hours). After another 18 hr. of stirring the mixture was cooled, acidified with 10% sulphuric acid, and extracted continuously with chloroform for three days. The chloroform extract was dried over anhydrous magnesium sulphate and evaporated leaving a thick sirup (130.2 mgm.). This product was methylated three times with Purdie's reagents after which no hydroxyl groups were detectable by infrared analysis. This fully methylated product (130 mgm.) in dry ether (10 ml.) was added, drop by drop, to a stirred suspension of lithium aluminum hydride (0.5 gm.) in anhydrous ether (25 ml.). After addition was complete the mixture was heated under gentle reflux for three hours. Excess lithium aluminum hydride was destroyed by cautious addition of ethyl acetate and, after acidification with 10% sulphuric acid, the mixture was filtered. The filtrate was freed of organic solvents by evaporation and the residual aqueous solution (25 ml.) was extracted continuously with ether for 24 hr. Evaporation of the dried (sodium sulphate) ether extract left a sirup (92.1 mgm.) of which an infrared spectrum showed the absence of a carbonyl group, which was present before reduction, and the presence of a hydroxyl group, which had not been found in the unreduced product. The reduced product was hydrolyzed by heating for six hours on a boiling water bath with 1% hydrochloric acid (2 ml.). The hydrolysis could not be followed polarimetrically because the solution was too highly colored. The hydrolyzate was neutralized with Dowex-2 (carbonate form) and examination by chromatography (solvent *D*) revealed the presence of 2,4-di-*O*-methyl xylose and 2,3,4-tri-*O*-methyl glucose. These two compounds were separated by preparative paper chromatography (solvent *D*) and extracted from the papers with acetone. The extracts were evaporated to sirups, which were taken up in water and filtered through charcoal. Evaporation of these clarified solutions left 2,4-di-*O*-methyl-D-xylose (15.2 mgm., $[\alpha]_D^{25} = +25.5$ ($c = 1.52$ in water)) reported (15) $[\alpha]_D^{25} = +24.7$; and 2,3,4-tri-*O*-methyl-D-glucose (14.0 mgm., $[\alpha]_D^{25} = +65.7$ ($c = 1.4$ in water)) reported (14) $[\alpha]_D^{20} = +65°$.

The 2,4-di-*O*-methyl-D-xylose was taken up in water (2 ml.) to which 0.5 ml. of bromine was added. The mixture was stored in the dark at room temperature for 48 hr., after which excess bromine was removed by aeration. The

clear solution was neutralized with Dowex-2 (carbonate form) and the neutral solution was evaporated to dryness. The residue was dissolved in methanol (5 ml.) and anhydrous ammonia was passed through the solution for one-half hour. After it was stored at 5°C. for 18 hr., the solution was evaporated to dryness leaving a semicrystalline residue which was recrystallized from ethyl acetate to yield the amide of 2,4-di-*O*-methyl-D-xylonic acid, m.p. 98–99°C., reported (15) m.p. 98–100°C.

The 2,3,4-tri-*O*-methyl-D-glucose was oxidized by heating at 90°C. for three hours with nitric acid (5 ml., sp. gr. 1.2). Nitric acid was removed by repeated distillation with water and methanol and the residue was esterified by ethereal diazomethane. Evaporation of the ethereal solution left a residue, which was extracted with ether. Removal of the ether gave a sirup which crystallized completely after four days at room temperature. The crystals, after trituration and washing with cold ether, had m.p. 105–106°C., reported (14) for the methyl ester of 2,3,4-tri-*O*-methyl-D-glucosaccharolactone, m.p. 106–107°C.

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THE VAPOR-PHASE PHOTOLYSIS OF ACETIC ACID[1]

By P. Ausloos[2] and E. W. R. Steacie

ABSTRACT

The photolysis of acetic acid (CH_3COOD) vapor has been investigated in the temperature range from room temperature to 285°C. Since CH_3D formation is independent of temperature, it is certain that the primary process

$$CH_3COOD+h\nu \rightarrow CH_3D+CO_2$$

occurs to the extent of about 10%. The results are complex and suggest that three other primary processes may occur, viz.

$$\begin{aligned} CH_3COOD+h\nu &\rightarrow CH_3CO+OD \\ &\rightarrow CH_3+COOD \\ &\rightarrow CH_3COO+D. \end{aligned}$$

The abstraction reaction

$$CH_3+CH_3COOD \rightarrow CH_4+CH_2COOD$$

is of importance, and the results indicate that it has an activation energy of 10.2 kcal., and a steric factor of the order of 10^{-3}.

INTRODUCTION

The photolysis of acetic acid in the gas phase was first investigated by Farkas and Wansborough-Jones (5), who suggested the primary processes:

$$CH_3COOH+h\nu \rightarrow CH_4+CO_2$$
$$(CH_3COOH)_2+h\nu \rightarrow CH_4+CO_2+CH_3COOH$$
$$(CH_3COOH)_2+h\nu \rightarrow C_2H_6+CO_2+CO+H_2O.$$

Burton (2) investigated the photolysis by mirror methods and suggested that radicals were formed by

$$CH_3COOH+h\nu \rightarrow CH_3+COOH,$$

followed by

$$COOH \rightarrow CO_2+H, \text{ etc.}$$

In later work (3) he rejected this mechanism and suggested that H-atoms but no CH_3 radicals were formed. He therefore proposed the primary step

$$CH_3COOH+h\nu \rightarrow CH_3COO+H.$$

Clusius and Schanzer (4) investigated the photolysis of CH_3COOD in the liquid phase, and concluded that two primary processes occur:

$$CH_3COOD+h\nu \rightarrow CH_3+COOD$$
$$CH_3COOD+h\nu \rightarrow CH_3D+CO_2,$$

the first process being the more important. They suggested that CH_4, found in the methane fraction, could be accounted for by the abstraction reaction

$$CH_3+CH_3COOD \rightarrow CH_4+CH_2COOD.$$

In the present work the photolysis of CH_3COOD has been investigated in the vapor phase over a more extended temperature range.

EXPERIMENTAL

The apparatus has been described previously (1). A Hanovia S-500 medium pressure mercury arc was used as a light source. The full radiation was used

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[2]*National Research Council of Canada Postdoctorate Fellow, 1952–54.*

to obtain the maximum intensity. The intensity was varied by the use of calibrated screens. Two samples of CH_3COOD were used, containing respectively 20% and 15% CH_3COOH. The analysis of the products was done by taking off two fractions, one at −196°C., and one at −150°C. These were then analyzed by a mass spectrometer. No attempt was made to determine condensable reaction products.

One experiment was made with a Corning 7-54 filter, with a cutoff below 2400 Å. This gave a rate approximately one quarter the normal rate. Since the filter has a transmission of about 40% at 2537 Å and decreases sharply below this, it appears that the effective radiation was mainly in the range from 2400 Å to 2537 Å.

RESULTS AND DISCUSSION

The results are given in Table I. The main reaction products found were CO, CO_2, CH_4, CH_3D, and C_2H_6. At temperatures above 150°C. H_2 and HD were also present, and they constituted about 10% of the reaction products at temperatures above 250°C. Very small amounts of CH_2D_2 and C_2H_5D were also detected. The amounts of these products were always less than two per cent of the methane or ethane fraction, and their formation is probably due to the presence of small amounts of CH_2DCOOD in the starting material.

The Primary Process

The results in Table I indicate that for runs at constant intensity the rate of formation of CH_3D is constant within experimental error, and is independent of temperature in the range from 27 to 287°C. On the other hand, the rate of formation of CH_4 increases steadily with increasing temperature as might be expected if it is formed in a secondary reaction. This constitutes a definite proof of the occurrence of the primary process

$$CH_3COOD+h\nu \rightarrow CH_3D+CO_2. \qquad [I]$$

If runs 9, 12, and 15 are compared it can be seen that the rate of formation of CO decreases sharply below 100°C., while at higher temperatures (compare runs 2–7) it is nearly independent of temperature. This suggests that CH_3CO is formed in the primary process

$$CH_3COOD+h\nu \rightarrow CH_3CO+OD, \qquad [II]$$

and decomposes thermally.

A comparison of CH_3D with CO_2 shows that primary process [I] accounts only for about 10% of the CO_2 formed. Nevertheless the rate of CO_2 formation is nearly independent of temperature over the whole range. This excludes the formation of CO_2 by a secondary process unless it is one with a very low activation energy. There appear to be two possibilities:

(*a*) $$CH_3COOD+h\nu \rightarrow CH_3+COOD \qquad [III]$$

followed by

$$COOD \rightarrow CO_2+D. \qquad [1]$$

TABLE I

Run	Temp., °C.	Pressure, cm.	Time, min.	Relative intensity	Products, cc./min. × 10^4							$k_8/k_7^{\frac{1}{2}} \times 10^{13}$ cm.$^{3/2}$ molecules$^{-\frac{1}{2}}$ sec.$^{-\frac{1}{2}}$	$\frac{2CH_4+2C_2H_6}{CO+CO_2-CH_3D}$
					CO	CO_2	CH_3D	CH_4	C_2H_6	H_2	HD		
Sample 1													
1	97	1.29	140	1	2.80	3.50	0.326	0.154	2.30			0.73	0.79
2	132	1.20	120	1	3.30	3.30	0.325	0.220	2.28			1.66	0.79
3	156	1.23	120	1	3.25	3.22	0.320	0.320	2.30			3.00	0.83
4	187	1.22	120	1	3.38	3.32	0.300	0.510	2.05	0.051	0.100	6.10	0.81
5	212	1.24	120	1	3.67	3.54	0.330	0.805	1.85	0.100	0.145	11.10	0.77
6	237	1.35	100	1	3.75	3.70	0.340	1.300	1.70	0.220	0.370	18.40	0.83
7	269	1.20	100	1	3.90	3.70	0.350	1.780	1.44	0.335	0.390	34.00	0.90
8	285	1.20	100	1.2	4.65	3.90	0.430	2.260	1.36	0.306	0.320	45.40	0.89
Sample 2													
9	27	1.51	100	1.28	1.33	5.30	0.410	0.142	2.13			0.44	0.73
10	28	1.50	200	0.58	0.57	2.36	0.195	0.075	0.88			0.34	0.70
11	27	1.48	1190	0.08	0.104	0.40	0.016	0.029	0.151			0.7	0.75
12	107	1.45	90	1.28	5.10	5.65	0.35	0.205	3.72			0.96	0.77
13	106	1.50	160	0.58	2.11	2.30	0.158	0.106	1.54			0.80	0.77
14	105	1.48	1080	0.08	0.287	0.345	0.021	0.034	0.225			0.87	0.85
15	232	1.45	80	1.30	6.15	5.98	0.405	1.78	2.51			19.8	0.73
16	236	1.53	135	0.08	2.17	2.52	0.196	1.21	0.91			21.7	0.94
17	168	1.28	100	0.85	2.65	3.00	0.223	0.355	1.58			4.55	0.71
18	169	1.32	200	0.35	2.14	1.25	0.100	0.230	0.76			4.30	0.60
19	168	1.35	1420	0.055	0.554	0.21	0.011	0.070	0.100	0.002	0.006	3.80	0.97
20	187	1.40	95	0.85	2.925	2.83	0.207	0.552	1.65	0.027	0.067	6.95	0.79
21	187	1.40	1156	0.055	0.194	0.187	0.018	0.108	0.09	0.0022	0.0045	6.10	1.11

This will only account satisfactorily for CO_2 production if [1] is very fast, which appears to be the case (8). The analogous reaction

$$COOCH_3 \rightarrow CO_2+CH_3 \qquad [2]$$

is known to occur readily (7). Reaction [1] will be less exothermic than [2], but is probably feasible thermochemically.

(*b*) $$CH_3COOD+h\nu \rightarrow CH_3COO+D \qquad [IV]$$

followed by

$$CH_3CO_2 \rightarrow CH_3+CO_2. \qquad [3]$$

In solution the acetate radical appears to have considerable stability (6), which casts some doubt on the rapid formation of CO_2 by [3] at low temperatures.

Either mechanism involves the formation of D-atoms. Since no D_2 is formed, and H_2 and HD are only formed at higher temperatures, recombination is apparently negligible compared with the abstraction reaction

$$D+CH_3COOD \rightarrow HD+CH_2COOD. \qquad [4]$$

H_2 will be formed from the CH_3COOH present in the acetic acid. The quantity of H_2 is, however, rather large compared with that of HD, and the possibility of the loss of an H-atom from the methyl group in the primary process is not excluded.

In any case the very small amount of H_2 and HD relative to CO_2 shows that most H- or D-atoms disappear in some other way to form condensable products, such as HDO and D_2O. The independence of CH_3D formation with temperature rules out the reaction

$$CH_3+D \rightarrow CH_3D. \qquad [5]$$

It is also possible to explain the above results in a somewhat simpler way by postulating the occurrence of reactions such as

$$CH_3COOD^*+CH_3COOD \rightarrow CH_3CO_2+CH_3CO+D_2O$$

or

$$(CH_3COOD)_2+h\nu \rightarrow CH_3CO_2+CH_3CO+D_2O.$$

It should be noted that at 27°C. under the experimental conditions used about 90% of the acetic acid is in the form of double molecules. At 150°C., however, the number of double molecules is negligible for the pressures used in this work. It should be pointed out that processes [I], [II], and [III] are nearly independent of temperature. It may therefore be concluded that double molecules do not affect the primary processes.

The Abstraction Reaction

The fact that the formation of CH_3D is essentially independent of temperature, while that of CH_4 increases rapidly with increasing temperature, indicates that only H-atoms on the methyl group are involved in abstraction reactions to an appreciable extent, viz.

$$CH_3+CH_3COOD \rightarrow CH_4+CH_2COOD. \quad [6]$$

If this is the only way in which CH_4 is formed, and if all C_2H_6 results from recombination,

$$2CH_3 \rightarrow C_2H_6, \quad [7]$$

then $$R_{CH_4}/R^{\frac{1}{2}}_{C_2H_6}[A] = k_6/k_7^{\frac{1}{2}},$$

where R_{CH_4} and $R_{C_2H_6}$ are the rates of formation of the products indicated and A represents acetic acid. In applying this relationship it is necessary to correct the CH_4 for the small amount formed by a process analogous to [I] from the small amount of CH_3COOH present.

The results given in Table I show that $k_6/k_7^{\frac{1}{2}}$ is not appreciably affected by a variation of the intensity by a factor of 15. The corrected values of $k_6/k_7^{\frac{1}{2}}$ are given in Fig. 1 in the form of an Arrhenius plot. A good straight line is

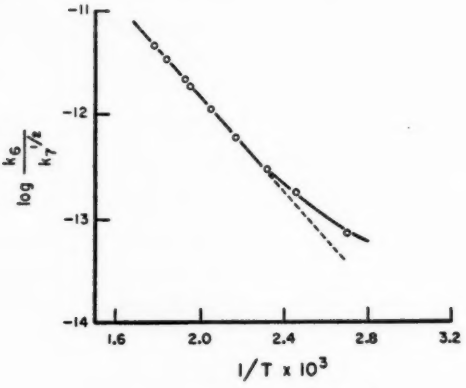

FIG. 1. Arrhenius plot of $k_6/k_7^{\frac{1}{2}}$.

obtained at higher temperatures with some curvature at low temperatures. The curvature may be connected with the presence of double molecules and wall reactions. The slope of the curve leads to a value of 10.2 kcal. for $E_6-\frac{1}{2}E_7 = E_7$. This is of the expected order of magnitude for such a reaction. The steric factor of reaction [7] is estimated to be $\sim 10^{-3}$.

Material Balance

On the basis of the above mechanism primary process [I] implies that

$$CH_3D = CO_2.$$

All other primary processes lead to $1CH_3$ for each CO_2 or CO formed. Hence it would be expected that

$$CO_2+CO-CH_3D = CH_4+2C_2H_6.$$

However, nothing has been said of the fate of the CH_2COOD radical formed

by abstraction. If it is assumed that this disappears only by recombination with methyl, then

$$(2CH_4+2C_2H_6)/(CO_2+CO-CH_3D) = 1. \qquad [A]$$

This ratio will be greater than unity if some CH_2COOD radicals disappear by dimerization, but will be low if CH_2COOD is formed by reaction [4] and is followed by the addition of methyl. A value of [*A*] somewhat less than unity is therefore to be expected. Any other processes leading to condensable products with elimination of CO or CO_2 will also lower the ratio.

The last column of Table I gives values of the ratio [*A*]. There is considerable scatter, but it will be seen that from 80 to 90% of the methyls are accounted for, which seems quite satisfactory in the absence of a detailed knowledge of the condensable products.

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REACTIONS OF ARYLSULPHONIC ESTERS

III. ON THE HYDROLYSIS OF METHYL *p*-METHYLBENZENESULPHONATE[1]

BY R. E. ROBERTSON

ABSTRACT

A study of the temperature dependence for the rate of hydrolysis of methyl *p*-methylbenzenesulphonate shows the specific heat of activation for this reaction in water to be 33.45±3 cal./mole degree. A comparison with the corresponding term for other methyl compounds reveals differences apparently characteristic of the anionic portion of the molecule. These differences are discussed in terms of specific solvation.

The possibility that the energy of activation for reactions in solution may be temperature dependent has been predicted and occasionally reported[2], but in general this property has been largely neglected. This is hardly surprising, since little interest was likely in such a derived quantity when the utility of the energy of activation was questionable (5, 6), especially since useful qualitative relationships, such as the S_N1–S_N2 classification, were more readily developed from a comparison of relative rates. Recently, Winstein, Grunwald, and Jones (16) and Swain and Scott (15) have suggested more quantitative classifications based on relative free energies. Such an approach, however, fails to take account of the many effects which result in compensating changes of energy and entropy. Nor will the substitution of the Arrhenius parameters or the equivalent energy and entropy of activation necessarily remove this objection unless it is known that the temperature dependence of the energy of activation is negligible.

Moelwyn-Hughes and co-workers (3, 10, 12) have shown that the specific heat of activation is by no means negligible for the hydrolysis of methyl halides and methyl nitrate. For these reactions ΔC_p[3] is not only large, but varies appreciably between the halides (10) and the nitrate, and may itself be temperature dependent. While it is probably too soon to assess the general significance of these results, and Moelwyn-Hughes has put forward two apparently unrelated hypotheses to account for the value he reports, it is clear that the magnitude of the ΔC_p term is such that it may not be neglected in any quantitative discussion relating structure and energy. In view of the prominent place given to ΔC_p values in discussion of the dissociation of weak acids, one may hope that the corresponding kinetic factor will prove a useful parameter in the quantitative study of reactions in solution.

Because of this possibility, a study was begun on the temperature dependence of the rate of hydrolysis for a series of benzenesulphonic esters. In this paper we report the results for methyl *p*-methylbenzenesulphonate.

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[2]*Theoretical and experimental results prior to 1935 are noted by LaMer, Ref. (7).*

[3]*The term ΔC_p indicates the specific heat of activation, as defined by the equation*

$$dE_A/dT = C_{act} - C_{int} = \Delta C_p$$

EXPERIMENTAL DETAIL

Water

Distilled water flowed through an 80 cm. × 35 mm. Acid–Base Ion Exchange column at the rate of 500 cc. per min. The first 600–800 cc. was rejected and the subsequent delivery used to make up solutions and rinse cells.

Methyl p-Methylbenzenesulphonate

Eastman Kodak white label methyl *p*-methylbenzenesulphonate was fractionally frozen to give material melting at 28.0–28.1°C.

Toluenesulphonic Acid

Eastman Kodak white label grade was used without further purification.

The Conductance Bridge

The conductance bridge was built about a Tinsley No. 4896 Electrolytic Conductivity Bridge. The 1000 cycle supply was from a Model 200 AB Hewlett-Packard Oscillator. This was passed through a phase shifter, opposite phases being supplied to the bridge through an isolating transformer, and to the X input of the detecting oscilloscope (Dumont-304H).

The output from the bridge passed through an isolating transformer (GR, 578A), a band pass filter (General Radio, 830-R), and a GR Amplifier (1231 B) and thence to the Y input of the oscilloscope. The Wagner ground of the Tinsley bridge was modified by the introduction of a Leeds and Northrup Decade Resistor (No. 4755) in series with the variable resistance, and balance was observed on a second oscilloscope between the high side of the output of the bridge and ground. This separate balancing was of particular advantage in following fast reaction rates and simultaneous reactions. All wiring was with RG-62U low-loss shielded cable and the leads to the bridge from the cells were carefully shielded, observing the usual precautions.

The Conductance Cells

In control experiments the first order rate was shown to be independent of the design of the cell, provided the resistance was of the proper order. One important restriction was that with more insoluble esters (8), certain precautions had to be taken to avoid the effects of adsorption of appreciable amounts of ester on the glass walls. The obvious precautions of avoiding cells with high surface to volume ratios and further of adopting the Guggenheim method (4) of determining the rate were usually sufficient. Shiny platinum or gold-plated platinum electrodes proved satisfactory. Platinum gray or black electrodes gave evidence of adsorption at low concentrations. While the presence of air did not alter the rate, it was standard practice to partially evacuate the cells to avoid separation of gas on the electrodes at higher temperatures.

Temperatures were determined by a Leeds and Northrup Mueller Bridge and a platinum resistance thermometer calibrated by the Heat Section of these laboratories. Temperatures were controlled within limits of ±0.003°C. Time was determined by either an electric clock driven by current from our

Standard Frequency Laboratory or for shorter runs by a split second-hand stop watch which was checked against the standard time.

EXPERIMENTAL PROCEDURE

After the cells were cleaned with hot chromic acid and repeatedly washed with distilled water, they were filled with a solution of toluenesulphonic acid of the same concentration used as supporting electrolyte in the solution to be studied. A solution containing ester (0.2 gm./l.) and toluenesulphonic acid (0.2 gm./l.) was then prepared. The cells were filled with this solution, evacuated, and sealed off. The presence of backing electrolyte reduced the relative change in electrolyte concentration during a run, and created a more reproducible environment. It was customary to preheat the cells in a subsidiary bath to the temperature under study, to avoid disturbing the main thermostat.

Resistances were measured at times t_1, t_2, t_3, etc., and $t_1+\sigma$, $t_2+\sigma$, $t_3+\sigma$, etc., where $\sigma = 1$ to 2 half-lives, and the slope of the plot of $\log[1/(R_{t_i}+\sigma) - 1/R_{t_i}]$ versus t_i gave the rate.

The use of this direct method involves the following approximation. Bolam and Hope (1) have shown that the equivalent conductance of benzenesulphonic acid in water is given by the equation

$$\Lambda = 383 - (0.227_4\ \Lambda_0 + 59.79)\ \sqrt{c} \qquad [1]$$

which may be rewritten for any particular experiment as

$$M/R_x = C - 0.38349\ C^{3/2} \qquad [2]$$

where M is a constant, characteristic of the system, and R_x is the measured resistance. If the concentration of backing electrolyte and ester is 1×10^{-3} molar at t_0 and hence 2×10^{-3} molar with respect to benzenesulphonic acid at t_∞ this will introduce a systematic positive error of approximately 1% in all the equivalent values. But the rate is derived from a difference of concentrations or (in the approximation) from the equivalent reciprocal ohms, so that the magnitude of this approximation is reduced to the order of 0.2%. The assumption that this argument applies equally at all temperatures and for similar strong acids does not seem unreasonable since the equivalent conductance of the proton is common to all. The application of this direct method was shown to give the same results, within the reproducibility possible, as those obtained using a calibration curve relating concentration and conductivity, and also was in agreement with values determined by our intermittent titration technique (13).

Precision

The errors introduced by fluctuations in temperature, time, and resistance readings were small compared to the errors arising as a result of what appears to be the adsorption of the ester on the glass walls of the cell. In Fig. 1 is shown a plot of the data for three typical runs done simultaneously. The resulting rates (37.04×10^{-5}, 37.14×10^{-5}, 36.72×10^{-5}) derived from these plots vary from the mean by 0.35% while the scatter of the points in

any run is estimated to be of the order of 0.1%. If care was taken that the concentration of ester did not fall below $5 \times 10^{-5}M$ during the run, this lack of reproducibility was reduced but probably not entirely eliminated by calculating rates by the Guggenheim method (4). With methyl esters this was easily achieved. With less soluble esters, errors from this source became the limiting factor. A further error for slow runs arose from what is generally called "glass-error" and rendered the data at 0° and 10°C. less reliable than the remainder.

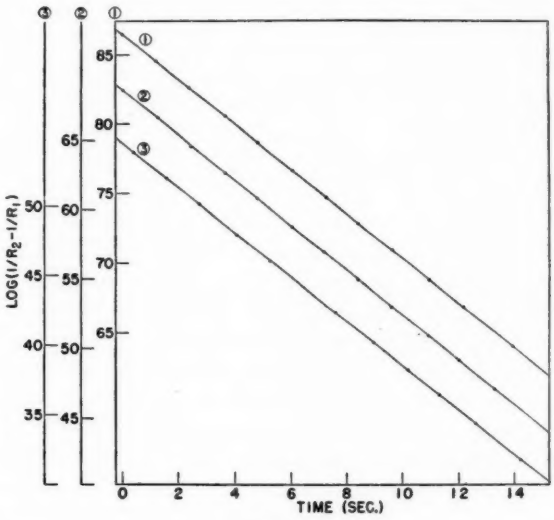

FIG. 1.

RESULTS

The initial concentrations of ester and the backing electrolyte were made up to 0.2 gm./l. in all experiments, and no attempt was made to explore salt effects (9), save for some preliminary experiments which indicated that a 10-fold increase in concentration of benzenesulphonic acid increased the rate by only a few per cent. Average rates corresponding to a series of temperatures are given in Table I, together with the average deviation from the mean, the number (n) of runs involved in the average, and the rate calculated from equation [4]. Prior to deriving such an equation by the direct application of the least mean squares to the data (average rates were used) it is advantageous to test the data for internal consistency by an adaptation of the graphical method of Everett and Wynne-Jones (2) based on equation [3],

$$[3] \qquad \Delta_{1,2}(T \log K)/\Delta_{1,2}(T) = (\Delta C_{p1,2}\, T \log T + B')/R\, \Delta_{1,2}(T),$$

by which these authors calculated the thermodynamic parameters from the ionization constants of weak acids, or more directly by the application of a plot of calculated E_A versus T such as was used by Moelwyn-Hughes (12). Our data

TABLE I

RATE DATA FOR THE HYDROLYSIS OF METHYL *p*-METHYLBENZENESULPHONATE IN WATER FOR A SERIES OF TEMPERATURES

Temperature, °C.	n	k_1 av.(sec.$^{-1}$) × 10^5 (obs.)		k_1 (sec.$^{-1}$) × 10^5 (calc.)
80.161	6	221	±3.5	221.8
75.216	4	147.5	±0.4	146.6
69.983	2	92.95	±0.1	93.05
65.128	5	60.1	±0.4	60.08
59.992	3	37.0	±0.2	37.15
55.894	4	25.05	±0.06	24.96
54.996	5	22.67	±0.12	22.84
50.008	4	13.81	±0.03	13.80
45.130	4	8.36	±0.05	8.27
40.142	4	4.789	±0.007	4.795
40.007	4	4.716	±0.017	4.724
35.138	5	2.71	±0.04	2.716
30.008	5	1.485	±0.007	1.481
24.850	4	0.778	±0.003	0.7839
20.009	4	0.421	±0.001	0.4208
14.998	3	0.2150	±0.007	0.2152
10.012	3	0.1082	±0.0002	0.1073
0.0621	4	0.02541	±0.00011	0.02438

plotted according to equation [3] are shown in Fig. 2. Where $\Delta_{1,2}T \backsim 10$°C., this plot provides a test of consistency, and on the basis of this test the data for 0° and 10°C. were omitted from the least mean square calculation. While the possibility that the systematic deviation noted in the rates for these temperatures may reflect some structural change in the solvent was considered, we believe that these data deviate from the remainder because of a larger glass-error resulting from the slowness of the runs. The slope of the curve in Fig. 2

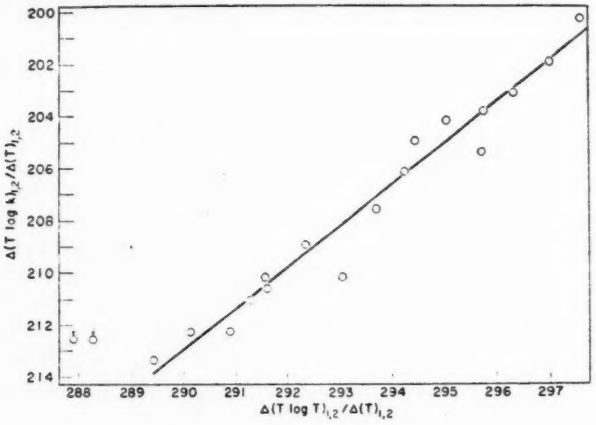

FIG. 2.

leads by the method of least mean squares to a value of ΔC_p of -33.9 ± 3 cal./deg. mole. The thermodynamic constants for equation [4] were then calculated by the method of least squares.[4]

[4]*See Hyne and Robertson. This issue.*

[4] $$\log_{10}k = (A/T)+B\log_{10}T+C$$

where
$$A = -\Delta H_0/2.3026\,R = -7036.861,$$
$$B = \Delta C_p/R = -16.8385,$$
$$C = \Delta S_0 - C_p/2.3026\,R = 60.1695,$$
$$0°\text{C.} = 273.16°\text{K. and } R = 1.9865,$$

[5] $$\Delta H_T = \Delta H_0+(-16.8385)RT.$$

Thermodynamic constants for the activation process at 298.16°C. are given in Table II.

TABLE II

THERMODYNAMIC CONSTANTS FOR THE ACTIVATION PROCESS AT 298.16°K.

ΔF	+6953.5 cal./mole
ΔH	−22220.4 cal./mole
ΔS	−51203 cal./mole deg.
ΔC_p	−33.45±3 cal./mole deg.

The error in ΔH is probably less than ±100 cal./mole. The random scatter of the points in Fig. 2 and the deviations between calculated and observed data in Table I show that the data are not sufficiently accurate to justify any claim for a change in ΔC_p with temperature in striking contrast to the results of Moelwyn-Hughes for the methyl halides (12).

DISCUSSION

In Table III, kinetic data are given for the hydrolysis of six methyl compounds. Large differences in the rates compared to that for methyl *p*-methylbenzenesulphonate are accompanied by corresponding changes in ΔH_T, but it is noted that the observed differences in this latter term would correspond to

TABLE III

A COMPARISON OF KINETIC DATA FOR HYDROLYSIS AT T = 303.19°K.

Compound	$k_1(\times 10^{-8}$ sec.$^{-1})$	$\Delta H_{303.19}$(kcal.)	ΔC_p	Notes
Me *p*–Me	1480	22.04	$33.4_7\pm3$	(*a*)
MeBr	84	26.36	(66)	(*b*)
MeI	17.3	28.31	(66)	(*b*)
MeCl	5.43	28.07	(66)	(*b*)
MeF	0.622	27.53	67	(*c*)
$MeNO_2$	0.201	29.300	42.6±14	(*d*)

Notes: (*a*) *This work.*
(*b*) *Ref. (11). The E_A values are recorded here. The ΔC_p values are merely approximate.*
(*c*) *Data calculated from equation [9], Ref. (3).*
(*d*) *Data calculated from equation [3], Ref. (9).*

rate differences from 2 to 700 times greater than actually found. It is interesting to note that the closest agreement on this basis (assuming constant entropy) is for the comparison with methyl fluoride, although the ratio of the rates is greater than 2000/1. The slope of the curve in Fig. 2 and the close agreement of the observed and calculated rates (Table I) leave no doubt that the activation energy for the hydrolysis of methyl *p*-methylbenzenesulphonate

changes with temperature in a systematic manner expressed by equation [5]. This change is of the same sign as, but of about one-half the magnitude of the values reported for the methyl halides in the lower temperature range, and there is no perceptible change in ΔC_p with temperature as was found for the halides.

Now it has long been recognized that the change in the bulk dielectric constant with temperature will lead to a corresponding change in the activation energy in the order of -13 cal./mole deg. for ionogenic reactions. It is clear that this factor will account neither for the magnitude of ΔC_p found for the above hydrolytic reactions, nor for the specific differences between compounds, nor would it be expected to lead to a difference in ΔC_p with temperature.

The fact that ΔC_p values for the hydrolysis of the methyl halides are roughly the same, in spite of widely differing bond strengths, led Moelwyn-Hughes to the conclusion that this coefficient is related to specific differences in the solvation of the reacting molecule. In view of the large difference between the sulphonate and the halides and to a lesser degree the nitrates, it seems probable that the specific solvation of the anionic part of the esters may play a major part in determining the value of this coefficient, and our explanation of the difference between the sulphonate ester and the corresponding halides is based on this postulate.

Reasoning on the basis of average specific heat values, Glew and Moelwyn-Hughes (3) calculate that the large and approximately equal ΔC_p values found for the hydrolysis of the methyl halides could be accounted for by the "freezing" of six water molecules (the freezing process for water involving a ΔC_p of -9 cal./mole deg.) about the transition state—the activation energy involving the breaking of water–water bonds. Such a model applied to the $\Delta C_p = -33$ cal./mole deg. found for the hydrolysis of methyl *p*-methylbenzenesulphonate would lead to a mechanism involving two water molecules reminiscent of the termolecular mechanism of Swain (14). If E_A is largely accounted for by the breaking of water–water bonds, as suggested in this hypothesis, the lower activation energy observed by us is not low enough to support such a hypothesis.

Qualitatively it is well known that the addition of a sulphonic group renders many organic molecules more soluble in water—an effect which points to strong interaction with the water of the initial state in hydrolysis of sulphonic esters. In attempting to account for the difference between the ΔC_p for the hydrolysis of the methyl halides and methyl *p*-methylbenzenesulphonate, in terms of solvation, it is reasonable to assume that the relative differences will be greater for the initial states where dipole–dipole interactions are weaker than for the transition states which involve partial charges. On this hypothesis, the lower value of ΔC_p reported for methyl *p*-methylbenzenesulphonate may be accounted for by assuming that the stronger solvation accompanying the initial state results in a smaller change in solvation on passing to the transition state as compared to the corresponding changes in solvation for the methyl halides. A similar explanation would also account for the lower value of ΔC_p

reported by McKinley-McKee and Moelwyn-Hughes (10) for the hydrolysis of methyl nitrate.

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ON THE TEMPERATURE DEPENDENCE OF THE REACTION VELOCITY[1]

By J. B. Hyne[2] and R. E. Robertson

ABSTRACT

The temperature dependence of the rates of three reactions in solution which the Arrhenius equation fails to express has been analyzed in terms of equations involving higher powers of the temperature and an improved fit of the experimental results obtained. The significance of the equations in terms of the thermodynamic parameters of the activation process is discussed.

There is a growing body of evidence to show that for important classes of reactions the Arrhenius equation fails to express fully the temperature dependence of reaction velocity. The Arrhenius equation assumes the activation energy to be temperature independent but this has not been fully tested in the majority of reactions in solution, since the temperature dependency has been derived from data over a limited temperature range with but an accuracy of a few per cent. With a few possible exceptions, such as in discussions of steric hindrance (3, 4), the activation energy has found little use save in converting rate data to a common temperature. Existing relationships between structure and the rate have been based on a comparison of rate data, the validity of such relations resting on the temperature independence of the Arrhenius parameters.

Both LaMer and Miller (15) and Moelwyn-Hughes and co-workers (8, 16, 17, 18), among others, have published results which indicate a marked temperature dependence of activation energy in particular systems. Neglect of such a measurable parameter at the present stage in the development of the kinetics of organic processes is not warranted and it is the object of this paper to present an analysis of kinetic data which considers the higher order temperature dependence of reaction velocity.

It must be recognized that the work of Harned *et al.* (11, 12), Everett and Wynne-Jones (7), and others (19, 23) on the analysis of acid and base dissociation constants as a function of temperature has already established a set of equations for the treatment of data for equilibrium systems. Accepting the assumption that the initial and transition states in a reaction are in equilibrium (5), such a system of equations may be applied to the analysis of rate–temperature data. The treatment of rate–temperature data in a manner analogous to the work on dissociation constants, however, presents problems peculiar to this field arising from difficulties attendant upon obtaining kinetic data of an accuracy comparable to e.m.f. data.

Refinement of the rate–temperature relationships for kinetic data would engender little interest were it not a necessary step in the long term problem of developing a more quantitative statement of the theory of the mechanism of reactions. A more immediate gain is the possibility that such refinement

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will provide a parameter (14) which can be interpreted in a thermodynamic sense and will provide a means of obtaining quantitative measurement of the factors involved in solvation processes accompanying reactions in solution; heretofore, such problems in solvation have been dealt with on an empirical basis (10, 22) by the study of reactions in mixed solvents or as a function of the bulk dielectric constant of the system (1).

TYPES OF EQUATION

Skrabal (21) has set down the various types of equation which have been suggested as representing the temperature dependence of rate data. Harned and Robinson (12) in particular, however, have treated the problem of progressive refinement of equations relating the equilibrium constants of dissociation processes and temperature and it is upon this work that our analysis of kinetic data is based. The Arrhenius equation

[1] $$\log k = (A/T)+B,$$

where $A = -E_a/2.303R$, assumes that the activation energy, E_a, is temperature independent. If the activation energy, or heat content change of the activation process ΔH, is expressed as a power series in T,

$$\Delta H = a+bT+dT^2,$$

the expression for the temperature dependence of the rate becomes

[2] $$\log k = (A/T)+B \log T+DT+C$$

where the thermodynamic functions of the activation process are given by

$$\Delta H = -2.303RA+BRT+2.303RDT^2,$$
$$\Delta C_p = RB+2.303R(2DT),$$
$$\Delta S = 2.303R(B \log T+2DT+C)+RB.$$

Harned and Robinson (12) and Everett and Wynne-Jones (7) have simplified this four-constant equation and have obtained two three-constant equations which, within the limit of accuracy of the data available to them for analysis, were found to represent the temperature dependence of the log k function studied.

[3] $$\log k = (A/T)+B \log T+C$$ (Everett and Wynne-Jones)

where
$$\Delta H = -R(2.303A-BT),$$
$$\Delta C_p = BR,$$
$$\Delta S = 2.303R(C+B \log T)+RB;$$

[4] $$\log k = (A/T)+DT+C$$ (Harned and Robinson)

where
$$\Delta H = -2.303R(A-DT^2),$$
$$\Delta C_p = 2.303R(2DT),$$
$$\Delta S = 2.303R(C+2DT).$$

The progressive refinement implied in equations [1] to [4] is seen when the thermodynamic parameters are expressed in terms of the constants of the

equations. Equation [1] as stated previously assumes ΔH to be temperature independent; equation [3] assumes a linear dependence of ΔH on temperature leading to a specific heat of activation term ΔC_p, which is temperature independent; equation [4] expresses ΔC_p as directly proportional to the absolute temperature; equation [2], the general case of [3] and [4], requires ΔC_p to be a linear function of the temperature. It is on the basis of equations [1] to [4] that the results of Robertson for the hydrolysis of methyl *p*-methylbenzenesulphonate (20), of LaMer and Miller for the dealdolization of diacetone alcohol (15), and of Moelwyn-Hughes for the hydrolysis of methyl chloride (18) have been analyzed.

CALCULATION

In Fig. 1 are shown plots of the activation energy against temperature for the three systems analyzed in this work. The data were analyzed by means

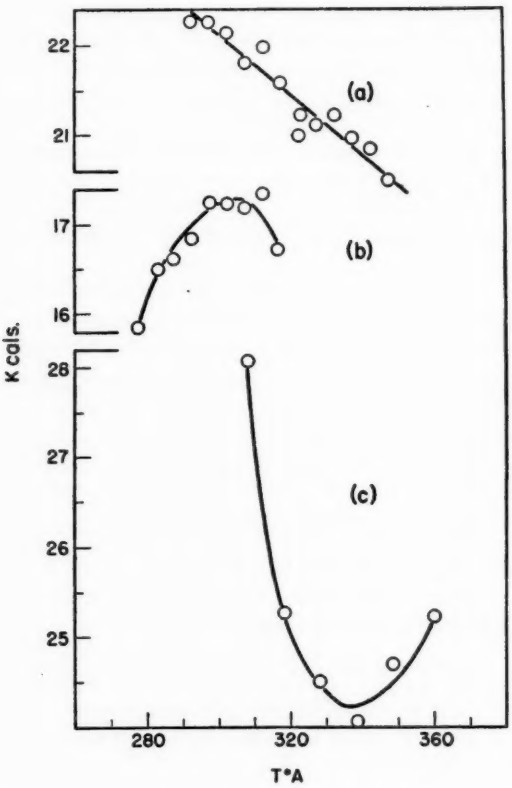

FIG. 1. Activation energy against temperature: (*a*) hydrolysis of methyl *p*-methylbenzenesulphonate; (*b*) dealdolization of diacetone alcohol; (*c*) hydrolysis of methyl chloride.

of the Arrhenius equation in the form [1′] using 10°C. temperature intervals, and the activation energies calculated for these intervals were associated with the mean temperature of the intervals:

$$[1'] \qquad \log(k_1/k_2) = E_a(T_1-T_2)/2.303R\ T_1T_2.$$

In the case of methyl *p*-methylbenzenesulphonate (Fig. 1(*a*)) the activation energy is a linear function of the temperature indicating a unique value of dE_a/dT over the temperature range studied, or in terms of thermodynamic parameters, a ΔC_p term which is temperature independent. In both the dealdolization of diacetone alcohol (Fig. 1(*b*)) and the hydrolysis of methyl chloride (Fig. 1(*c*)) the activation energy is a non-linear function of temperature indicating that the ΔC_p terms are temperature dependent. The curvature of the plot over the temperature range available is more marked in the case of methyl chloride than in that of diacetone alcohol hinting that the temperature dependence of the ΔC_p term is more complex in the former case. Such preliminary plots in the detailed analyses of rate–temperature data serve to indicate, in addition to the nature of the ΔC_p temperature dependence, any determinations which are not internally consistent with the other data since they will appear as deviating widely from the general trend of neighboring points. Calculation of the activation energy at intervals of less than 10°C. requires exceptionally accurate experimental data and is not necessary for preliminary plots such as in Fig. 1 when a temperature range of 40 to 50 degrees is available for study.

Figs. 2, 3, and 4 show, for the three systems studied, the deviations between observed and calculated log k values after the manner of Pitzer (19). The rate–temperature data for each system have been analyzed in terms of the various equations set down in the previous section until a minimum deviation between the observed log k value and that calculated from the equation fitted was obtained. The constants of the various equations were determined by a least mean square fit method applied directly to the data except in the case of the four-constant equation where an empirical graphical correction was applied to obtain the fourth constant C. The least mean square method used was that outlined by Goulden (9) using the method of Gauss Multipliers.

In work where rates of reaction of several systems are determined at a fixed series of temperatures the Gauss Multiplier method has much to recommend it since the Gauss Multipliers are dependent only on the temperature data and once calculated can be applied to any set of rate data determined for that particular set of temperatures.

In the design of experiments the extent of the temperature range studied is of considerable importance. In the application of the least mean square method to the results the inclusion of one determination at a temperature 10°C. outside the initially studied range (say 10° to 60°C.) is equivalent to increasing the number of determinations within the range by a factor of 1.7. The importance of determinations at the extremes of the temperature range has been pointed out previously (2) and great care is necessary in establishing the accuracy of these points.

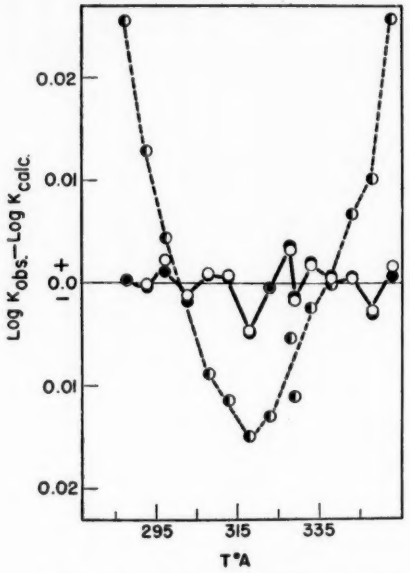

FIG. 2. Methyl *p*-methylbenzenesulphonate.

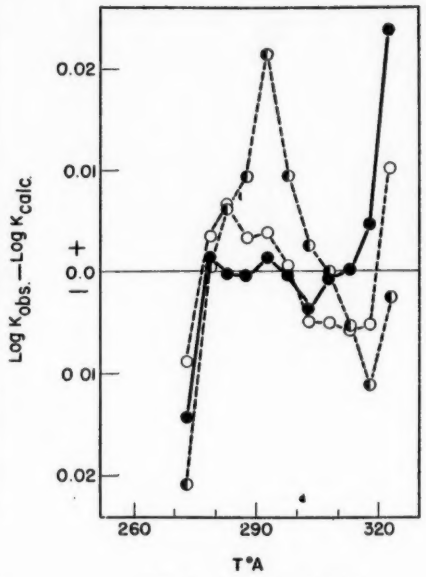

FIG. 3. Diacetone alcohol.

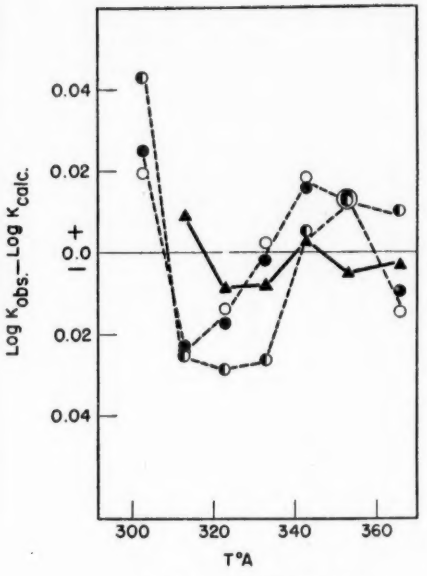

FIG. 4. Methyl chloride.

◐ $\log k = (A/T)+B$
○ $\log k = (A/T)+B \log T+C$
● $\log k = (A/T)+DT+C$.
▲ $\log k = (A/T)+B \log T+DT+C$

The equations derived for the three systems by the method of least mean squares were:

Methyl p-methylbenzenesulphonate (*Fig.* 2)

[1*a*] $\log k = (-4706.257/T)+10.6902$

[3*a*] $\log k = (-7036.861/T)-16.8385 \log T+60.1695$

[4*a*] $\log k = (-5885.250/T)-0.0115793\ T+18.0932$

Diacetone alcohol (*Fig.* 3)

[1*b*] $\log k = (-3681.667/T)+10.7035$

[3*b*] $\log k = (-1874.496/T)+14.0194 \log T-30.0565$

[4*b*] $\log k = (-2571.561/T)+0.0129521\ T+3.1090$

Methyl chloride (*Fig.* 4)

[1*c*] $\log k = (-5492.118/T)+10.8925$

[3*c*] $\log k = (-8708.034/T)-22.2727 \log T+76.7500$

[4*c*] $\log k = (-6739.194/T)-0.0112566\ T+18.4003$

[2*c*] $\log k = (-4474.950/T)+7.0453 \log T-0.001067\ T-9.5704$

DISCUSSION

Methyl p-Methylbenzenesulphonate

The failure of the Arrhenius equation [1*a*] to represent the data is clearly shown in Fig. 2, a systematic deviation between the observed and calculated log k values being observed. Analysis of the data in terms of equations [3*a*] and [4*a*] resulted in a random scatter of the deviation about the zero line in Fig. 2 showing that it is impossible to distinguish between the accuracy of fit of the two equations. Both equations take into consideration temperature dependence of the activation energy, i.e. they include a ΔC_p term, equation [3*a*] assuming ΔC_p to be temperature independent and [4*a*] requiring ΔC_p to be directly proportional to the absolute temperature. As Harned and Robinson (12) have pointed out, the fact that both equations fit the data equally well indicates that any temperature dependence of ΔC_p must be very small. The best fit of equations [3*a*] and [4*a*] also agrees with the linearity found in the E_a/T plot of Fig. 1(*a*).

Diacetone Alcohol

As in the methyl *p*-methylbenzenesulphonate system the failure of the Arrhenius equation [1*b*] is seen in Fig. 3. In this system, however, somewhat better agreement between observed and calculated log k values is found for equation [4*b*], where allowance is made for ΔC_p being directly proportional to the absolute temperature, compared with equation [3*b*], where the ΔC_p is assumed to be temperature independent. The preliminary plot of E_a against T established the non-linearity of the temperature dependence of E_a and hence the temperature dependence of the corresponding ΔC_p term. Inspection of the deviation curves in Fig. 3 shows that even the three-constant equation [4*b*], allowing temperature dependence of ΔC_p, does not give good agreement between the observed and calculated log k values especially at the limits of the temperature range employed. It does, however, provide evidence of the existence of data for a system exhibiting temperature dependence of ΔC_p.

Methyl Chloride

Fig. 4 again illustrates the failure of the Arrhenius equation [1*c*] to express the temperature dependence of the rate of hydrolysis of methyl chloride. In this system, however, although the two three-constant equations [3*c*] and [4*c*] give a better fit, the deviation between observed and calculated log k values is still systematic and not random. The four-constant equation [2*c*], which considers a linear dependence of ΔC_p on temperature (but not direct proportionality), gives a still better fit with all points except that at the lowest temperature. Furthermore, it should be noted that the coefficient of the log T term in [2*c*] has changed sign compared with that in the three-constant equation [3*c*] resulting in a change in sign of the ΔC_p term. The reason for this can be seen by considering the effect of neglecting the lowest temperature determination in Fig. 1(*c*) which will result in the slope of the best straight line through the points changing from negative to positive, and hence the change of sign of the ΔC_p. Investigation of the form of the systematic deviations between the observed and calculated values of log k for the methyl chloride system has shown that an improved over-all fit would require an equation involving at least a cubic term in T. Calculation of the constants of such an equation is an extremely laborious process and even with such an equation the physical significance of the coefficients of the higher powers of T could not be established on the basis of the present theory. The apparent complexity of the equation required to express the temperature dependence of rate in this case suggests the possibility that the hydrolysis of methyl chloride is not a simple reaction but is accompanied by a parallel reaction having a different activation energy (13). Moelwyn-Hughes does not consider this possibility in his presentation of the data although he offers several possible suggestions as explanations of the complex temperature dependence of the activation energy. Confirmation of the data, particularly at the extremes of the temperature range, is obviously desirable. The methyl chloride example is paralleled by the results for methyl bromide and methyl iodide also given by Moelwyn-Hughes (18) but not analyzed here.

The importance of choice of equation when the fit is not good is illustrated in Table I by a comparison of the ΔC_p terms calculated for the various systems

TABLE I

Equation	$\Delta C_p^{313.16}$ (cal./deg. mole)		
	Methyl *p*-methylbenzenesulphonate	Diacetone alcohol	Methyl chloride
[3]	−33.5	−27.9	−44.2
[4]	−33.2	−37.1	−32.2
[2]			+10.4

from the corresponding coefficients of the equations used. For methyl *p*-methylbenzenesulphonate, where both equations [3*a*] and [4*a*] fit the data within the experimental error, the ΔC_p values are in excellent agreement. The diacetone alcohol system, where the fit is somewhat poorer, gives two

values of ΔC_p differing by 10 cal./deg.mole, while in the methyl chloride system, where the fit is poor even in the case of the four-constant equation [2*c*], the ΔC_p value calculated by the four-constant equation [2*c*] is of opposite sign to those evaluated from equations [3*c*] and [4*c*]. In no case, it should be noted, does the value of the ΔC_p determined by a least mean square method agree with the value quoted by Moelwyn-Hughes (17).

These systems suggest that kinetic data may well require more complex expressions to describe their temperature dependence than the corresponding dissociation constant temperature dependence relationships where Harned and Robinson (12) and Everett (6) found no evidence for discriminating between equations of the type [3] and [4]. Whether this apparent necessity for more complex equations to account for rate–temperature behavior is a reflection of a more complex activation process or is due to the fact that systems exhibiting complex temperature dependence of dissociation constants have not as yet been studied, is a question which only further work in both fields will answer.

ACKNOWLEDGMENTS

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SOME CORRELATIONS OF THE MOLECULAR STRUCTURE OF ORGANIC PHOSPHORUS COMPOUNDS WITH THEIR INFRARED SPECTRA[1]

By R. B. Harvey[2] and J. E. Mayhood[3]

ABSTRACT

The infrared spectra of fourteen organic esters of phosphorus containing dimethylamino groups, and two esters containing diethylamino groups have been examined over the region 5000 to 670 cm.$^{-1}$ (2 to 15 microns). Correlations have been obtained between absorption band frequencies and the presence of dialkylamino groups in the molecule as an aid to their identification in the spectra of phosphorus compounds. A number of compounds of the pyrophosphate type have been examined and absorption associated with the (P—O—P) linkage has been found in the 950 to 910 cm.$^{-1}$ (10.5 to 11.0 micron) region. Some criteria are proposed to distinguish absorption by the (P—O—P) linkage in this region.

INTRODUCTION

Some convenient method for the identification of compounds or of specific groupings present in compounds is desirable either from the standpoint of process or quality control in industrial application, or for the chemist in laboratory operations. Since the development of the theory of infrared spectra is not yet adequate to interpret the observations without accessory data except in the simplest cases, a system of correlations is required between known molecular configurations and absorption spectra. The present paper is concerned with such a series, characteristic of the dimethylamino (Me_2N) group in esters of phosphorus compounds. Some limited data are presented on absorption bands attributed to the (P—O—P) bond.

EXPERIMENTAL

A Perkin-Elmer Model 21 double beam recording spectrophotometer was used to obtain the spectra presented in Figs. 1 to 19. The particular instrument is set up to scan on a linear wavelength scale, but all references to absorption bands have been converted to wave numbers (cm.$^{-1}$). The absorption of energy was read in units of optical density or absorbance; the ordinate of the absorption curves in the figures is labelled "% transmission", the origin being 0% and the topmost index line being 100% transmission. Most of the samples were run as contact films; any exceptions to this are noted on the spectra concerned. Since many of the compounds are highly hygroscopic, care was taken to eliminate moisture both from the windows and from the cell surfaces coming into contact with the samples, and by preparing the samples in a dry box or under the beam of an infrared lamp. The presence of (OH) bands is usually a fairly good test of water that may have been present in the sample or that may have been picked up during sample preparation. Water bands in the majority of the spectra were of negligible importance. Spectra were obtained at room temperature.

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Contribution from the Physics Section, Suffield Experimental Station, Ralston P.O., Alberta.
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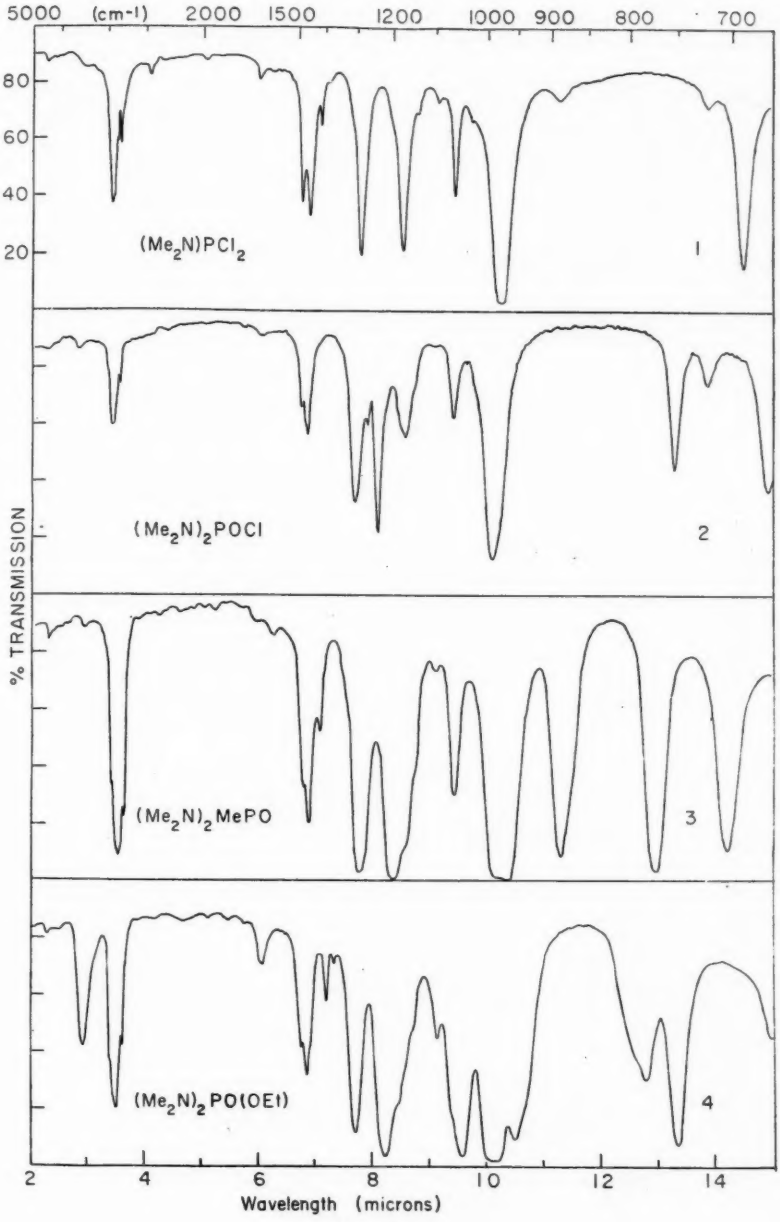
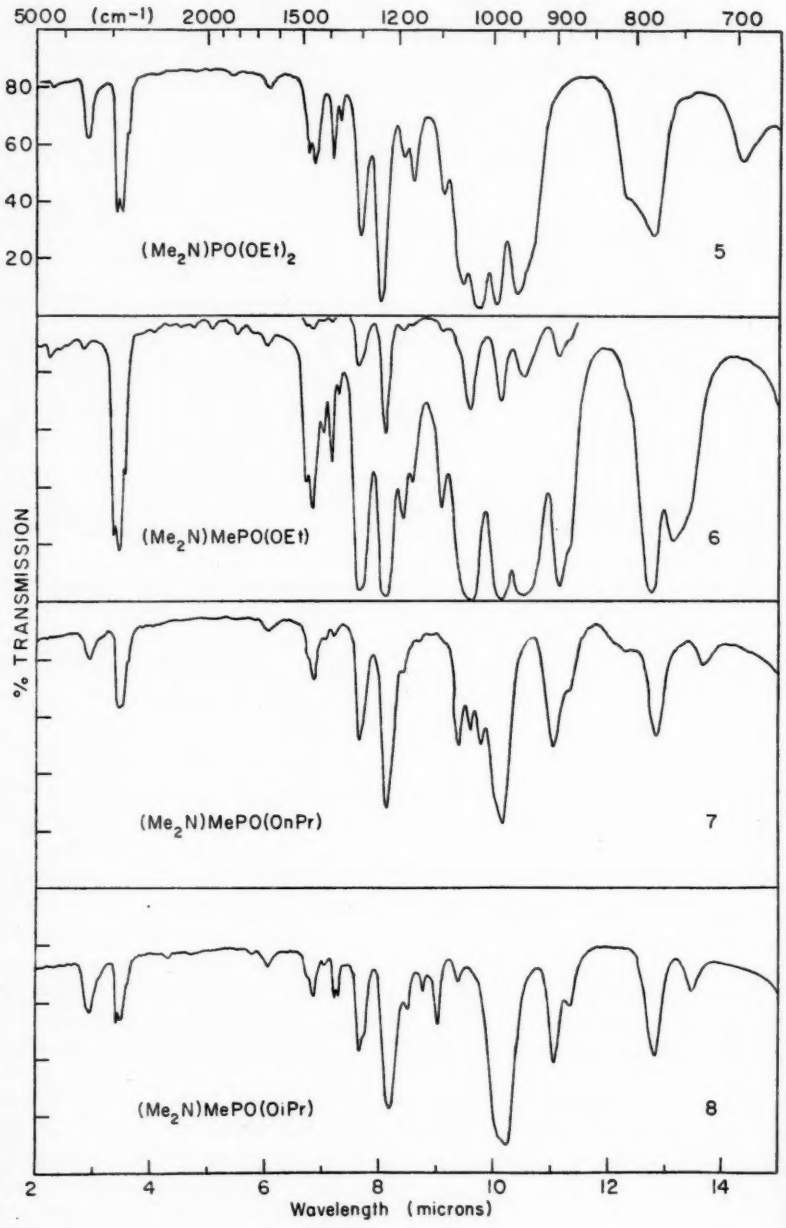
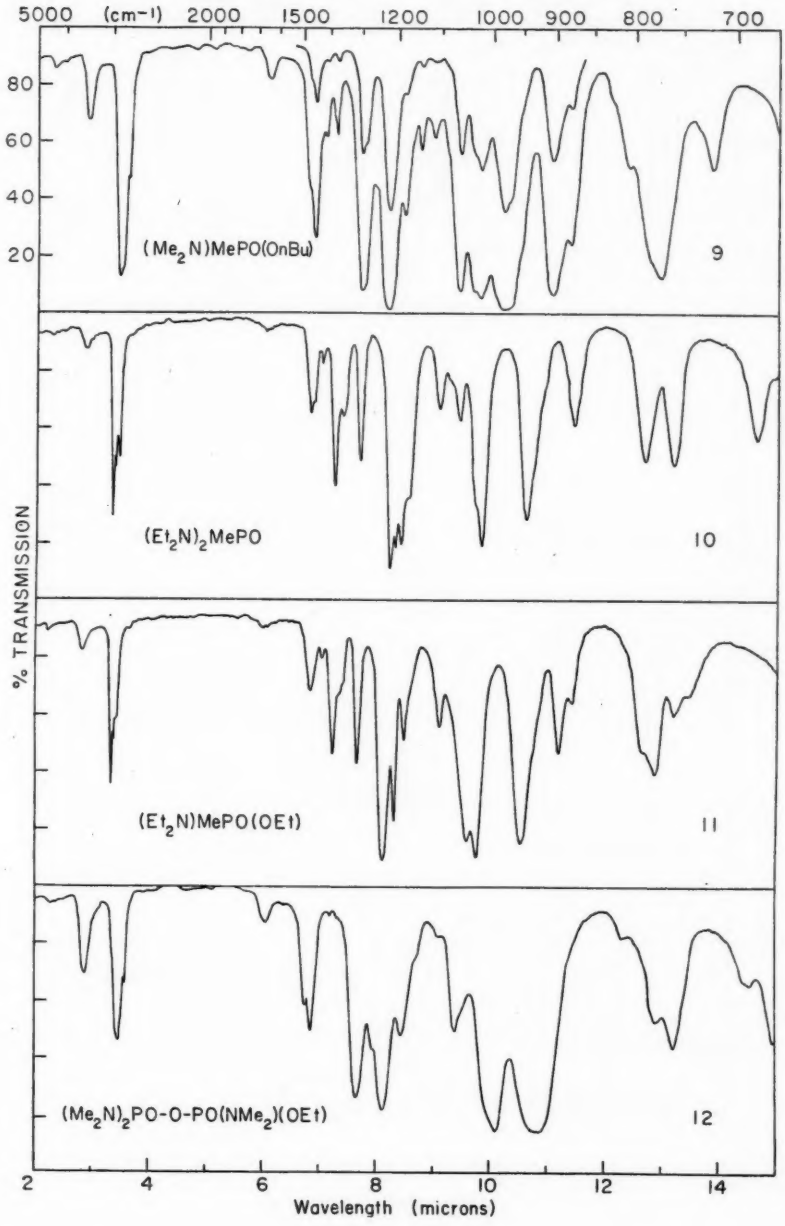

5000 (cm-1) 2000 1500 1200 1000 900 800 700
80
60
40
20
(Me2N)PCl2
1
(Me2N)2POCl
2
(Me2N)2MePO
3
(Me2N)2PO(OEt)
4
% TRANSMISSION
2 4 6 8 10 12 14
Wavelength (microns)

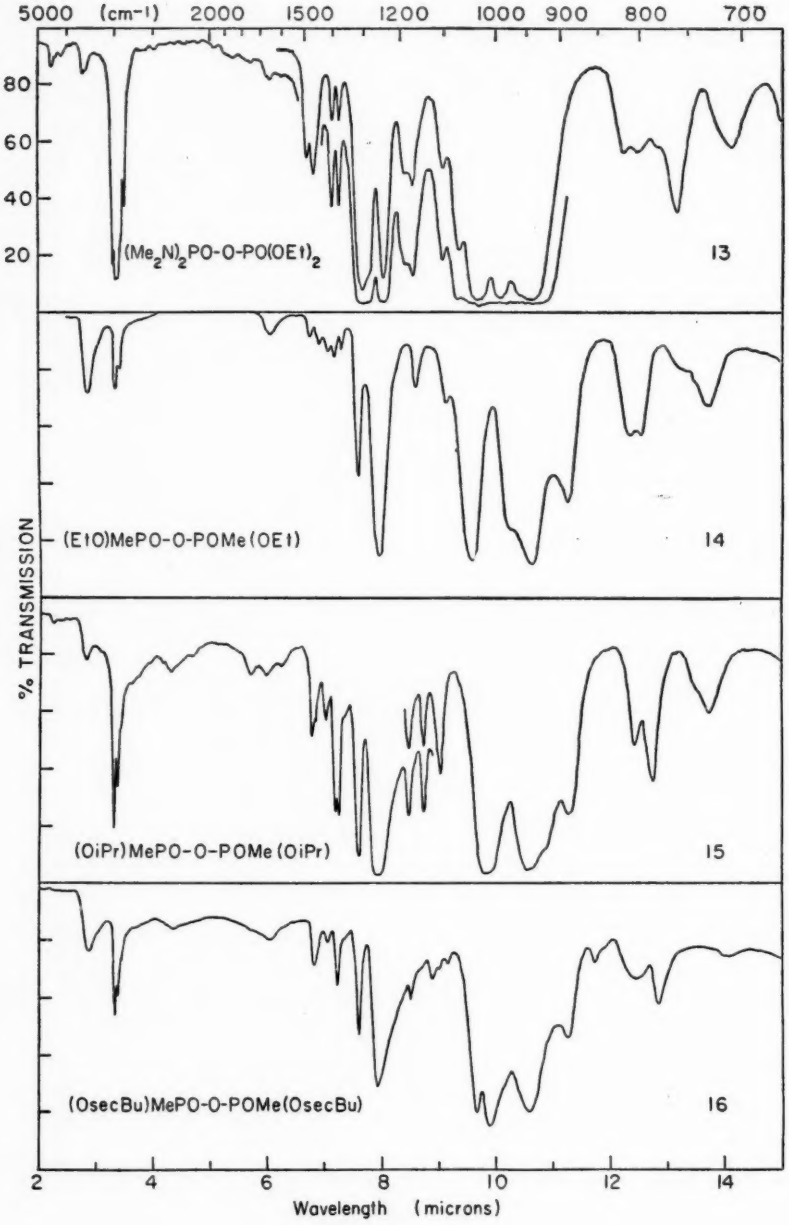
FIGS. 1 to 4.


5000 (cm-1) 2000 1500 1200 1000 900 800 700
80
60
40
20
(Me2N)PO(OEt)2
5
(Me2N)MePO(OEt)
6
% TRANSMISSION
(Me2N)MePO(OnPr)
7
(Me2N)MePO(OiPr)
8
2 4 6 8 10 12 14
Wavelength (microns)

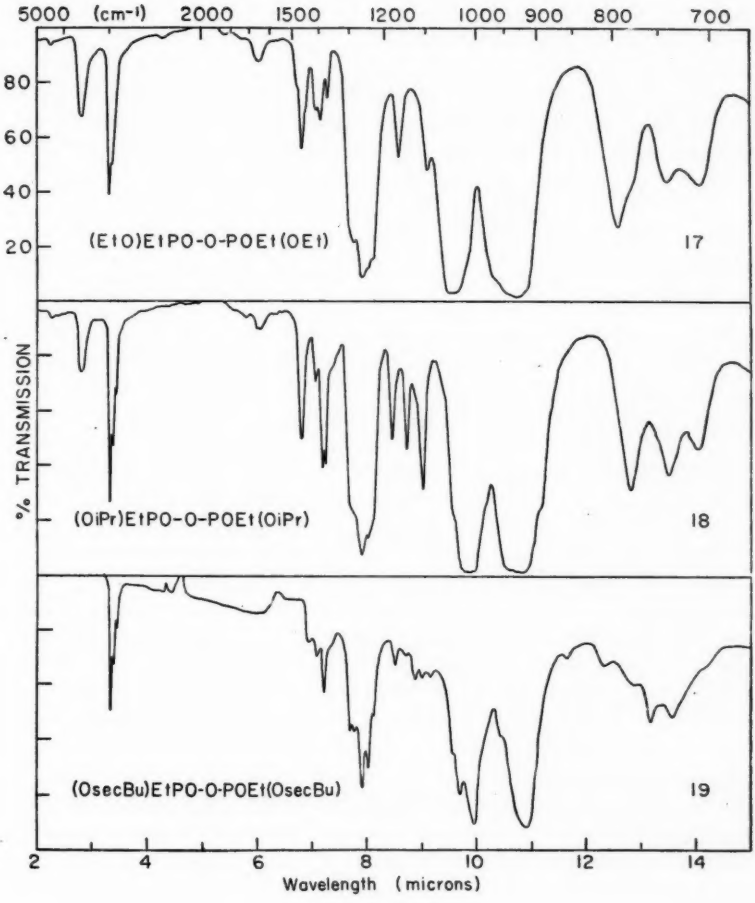
FIGS. 5 to 8.


5000 (cm-1) 2000 1500 1200 1000 900 800 700
80
60
40
20
(Me2N)MePO(OnBu)
9
(Et2N)2MePO
10
(Et2N)MePO(OEt)
11
(Me2N)2PO-O-PO(NMe2)(OEt)
12
% TRANSMISSION
2 4 6 8 10 12 14
Wavelength (microns)

FIGS. 9 to 12.


5000 (cm-1) 2000 1500 1200 1000 900 800 700
80
60
40
20
% TRANSMISSION
(Me2N)2PO-O-PO(OEt)2
13
(EtO)MePO-O-POMe(OEt)
14
(OiPr)MePO-O-POMe(OiPr)
15
(OsecBu)MePO-O-POMe(OsecBu)
16
2 4 6 8 10 12 14
Wavelength (microns)

FIGS. 13 to 16.


5000 (cm-1) 2000 1500 1200 1000 900 800 700
80
60
40
20
% TRANSMISSION
(EtO)EtPO-O-POEt(OEt)
17
(OiPr)EtPO-O-POEt(OiPr)
18
(OsecBu)EtPO-O-POEt(OsecBu)
19
2 4 6 8 10 12 14
Wavelength (microns)

FIGS. 17 to 19.

Most of the compounds whose spectra are presented were prepared through the co-operation of the Chemistry Section of the Suffield Experimental Station. Redistilled materials were used, and where possible, separate preparations of the same compound were examined for deviations in the spectrum. The spectrum of any compound of doubtful purity is marked. In Table I are listed compounds that we are concerned with in this paper, together with their boiling points and refractive indexes. Formulas are given for compounds to avoid confusion in the nomenclature.

TABLE I*

COMPOUNDS EXAMINED BY INFRARED METHODS FOR STRUCTURAL CORRELATIONS

Compounds	Figure number	Boiling point, °C./mm.	Refractive index, °C./index	Remarks
$(Me_2N)PCl_2$	1	41/12.5	—	Chlorine: calc. 48.5%; found 48.42%
$(Me_2N)POCl_2$	—	88/25	22/1.4634	Chlorine: calc. 43.8%; found 44.0%
$(Me_2N)_2POCl$	2	76/2.5	28/1.4635	
$(Me_2N)_2MePO$	3	60.5/0.6	26.5/1.4560	
$(Me_2N)_2PO(OEt)$	4	59/1	24/1.4343	
$(Me_2N)PO(OEt)_2$	5	64/3	24/1.4175	
$(Me_2N)MePO(OEt)$	6	55/1	—	
$(Me_2N)MePO(OnPr)$	7	62.2/1	25.5/1.4320	
$(Me_2N)MePO(OiPr)$	8	42.5/0.04	22/1.4300	
$(Me_2N)MePO(OnBu)$	9	64/0.5	26/1.4341	
$(Et_2N)_2MePO$	10	91/1	21/1.4580	
$(Et_2N)MePO(OEt)$	11	54/1	21/1.4360	
$(Me_2N)_2PO—O—PO(NMe_2)_2$	—	82–83/10^{-3}	24/1.4620	
$(Me_2N)_2PO—O—PO(NMe_2)(OEt)$	12	—	20/1.4564	Purity not checked
$(Me_2N)(OEt)PO—O—PO(OEt)(NMe_2)$	—	—	18/1.4300	Purity not checked
$(Me_2N)_2PO—O—PO(OEt)_2$	13	101–103/5×10^{-4}	25/1.4380	
$(EtO)MePO—O—POMe(OEt)$	14	68–70/3.5×10^{-4}	20/1.4360	Analysis for carbon and hydrogen for all oxygen compounds checks well with theory
$(OiPr)MePO—O—POMe(OiPr)$	15	77–80/0.07	25/1.4270	
$(O\textit{sec}Bu)MePO—O—POMe(O\textit{sec}Bu)$	16	86/0.07	23/1.4329	
$(EtO)EtPO—O—POEt(OEt)$	17	70/7×10^{-4}	25/1.4342	
$(OiPr)EtPO—O—POEt(OiPr)$	18	65/6×10^{-4}	25/1.4304	
$(O\textit{sec}Bu)EtPO—O—POEt(O\textit{sec}Bu)$	19	85–87/0.07	20/1.4391	
$(EtO)(EtO)PO—O—PO(OEt)(OEt)$	—	110/0.05	25/1.4177	

**The compounds in this table were supplied by the Chemistry Section of the Suffield Experimental Station. Their data are quoted for boiling points, refractive indexes, and analytical results.*

RESULTS AND DISCUSSION

(a) *The Dimethylamino Grouping* (Me_2N)

Interest in the dimethylamino grouping, $\left[N\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}\right]$, has arisen from the compound Schradan, in which all the ethoxy groups (EtO) of tetraethyl pyrophosphate have been replaced by dimethylamino groups. Many phosphonic acid esters containing the dimethylamino group have been synthesized and spectra have appeared in some publications. Bellamy and Beecher (2) and Colthup (4) have ascribed an absorption band at about 7.8 μ (1280 cm.$^{-1}$) to secondary aromatic amines. Holmstedt and Larrson (11) suggest that bands at 9.94 to 10.11 μ (1006 to 989 cm.$^{-1}$) and 13.7 to 14.25 μ (730 to 702 cm.$^{-1}$) are characteristic of dimethylamino compounds. They suggest the latter absorption may be the result of stretching vibrations of the (P—N) bond in the combination $\left[P{-}N\begin{smallmatrix}C\\C\end{smallmatrix}\right]$; the former they suggest may be associated with vibration in the (C—N) bond. Corbridge and Lowe (5) have calculated the absorption wavelength to be expected from a (P—N) stretching vibration by Gordy's relation (9) to be about 13.5 μ (740 cm.$^{-1}$). This is in agreement with their observation of a strong absorption near 14.3 μ (700 cm.$^{-1}$) in each of their phosphoramidates. Absorption in the 6.18 and 6.8 μ (1620 and 1470 cm.$^{-1}$) regions may be ascribed to zwitterion formation in this type of compound.

The spectra of 13 of the 16 compounds considered are presented here, 11 of them containing the dimethylamino group, and 2 containing the diethylamino group (see Figs. 1 to 13). Some characteristic absorption bands of the compounds are assembled in Table II. Compounds containing alkyl hydrocarbon groups usually exhibit absorption from 3130 to 2860 cm.$^{-1}$, which is the region attributed to the (C—H) stretching vibrations. Absorption arising from dimethylamino groups appears in a narrow band from 2850 to 2780 cm.$^{-1}$. The absorption band in this region is usually rather weak and may consist of a subsidiary peak or shoulder. The dimethylamino group appears to absorb at 2910 and 2890 cm.$^{-1}$ respectively in the two compounds.

Absorption in the region of 1470 cm.$^{-1}$ appears to be enhanced when dimethylamino groups are attached to phosphorus. A comparison of the spectra of compounds containing the dimethylamino group with the spectra of similar compounds without the group indicates a predominant absorption peak at 1460 to 1450 cm.$^{-1}$. Absorption is almost continuous over the region 1480 to 1430 cm.$^{-1}$ for the compound $[(Me_2N)(EtO)PO]_2O$. The two compounds containing diethylamino groups exhibit absorption in the region 1390 to 1350 cm.$^{-1}$, rather than at 1470 to 1450 cm.$^{-1}$, considerably greater than is normally observed for ethyl esters of phosphonic acids—compare for instance, the spectrum of tetraethyl pyrophosphate.

Compounds containing the methyl phosphoryl group (Me—P=O) exhibit a single sharp absorption band at about 1308 cm.$^{-1}$ with a width at half

TABLE II

CORRELATION OF ABSORPTION BANDS WITH (NMe_2) GROUPS IN SOME PHOSPHORO-ESTERS

Compound	Figure number	2860–2780 cm.$^{-1}$ region	1470 cm.$^{-1}$ region	1320–1280 cm.$^{-1}$ region	1190 cm.$^{-1}$ region	1070 cm.$^{-1}$ region	1000–980 cm.$^{-1}$ region	710–670 cm.$^{-1}$ region
$(Me_2N)PCl_2$	1	2840 2790	1478 1450	1287 (25)*	1175	1063	978	690 s
$(Me_2N)POCl_2$	—	2820	1486 1468	1315 (33)	1175	1070	994	723 s
$(Me_2N)_2POCl$	2	2820	1485 1468	1307 (36)	1175	1068	995	755 m 672 m
$(Me_2N)_2POMe$	3	sh 2800 2760	1480 1460	1300 (36)	1184	1065	988 970	704 s
$(Me_2N)_2PO(OEt)$	4	2750	— 1460	1303 (34)	1184	sh 1070	989	665
$(Me_2N)PO(OEt)_2$	5	2760	1458 1450	1308 (22)	1190	1062	998	698
$(Me_2N)POMe(OEt)$	6	2810	1465 sh 1450	1303 (33)	1188	sh 1068	988	660
$(Me_2N)POMe(OnPr)$	7	2770	1460 sh 1440	1308 (34)	1188	1067	sh 999 985	660 w
$(Me_2N)POMe(OiPr)$	8	2760	1458 sh 1448	sh 1295 (41)	1178	1067	sh 994 980	662 w
$(Me_2N)POMe(OnBu)$	9	2770	— 1462	1305 (33)	1190	1067	986 sh 975	660 mw
$(Et_2N)_2POMe$	10	2890	1472 1459	1305 (16)	1192 1177	1065	—	684 m
$(Et_2N)POMe(OEt)$	11	2910	1467 sh 1453	1310 (16)	1184	sl sh 1065	—	662 mw
$(NMe_2)_2PO—O—PO(NMe_2)_2$	—	2810	— 1466	1310 (36)	1188	1070	992	668 s
$(NMe_2)_2PO—O—PO(NMe_2)(OEt)$	12	2790	— 1460	1310 (41)	1188	1068	993	668 mw
$(NMe_2)(OEt)PO—O—PO(NMe_2)(OEt)$	—	2790	1460 1448	Very broad	1188	Very broad	982 (absorption from 900 to 1100 cm.$^{-1}$)	Not clear
$(NMe_2)_2PO—O—PO(OEt)_2$	13	2850	— 1468	1305 Very broad Several peaks merged	1190	1070	994	710 m 667 w

The figures in brackets indicate the width of the absorption band at half the maximum height of the peak.

height ($\Delta\nu_{1/2}$,)* of about 16 cm.$^{-1}$. Absorption is shown in the region 1320 to 1280 cm.$^{-1}$ by compounds having a dimethylamino group, and if (Me—PO) and (Me_2N) groups both are present, the sharp absorption band of the former is obscured. Measurement of $\Delta\nu_{1/2}$ in such compounds gives values of 33 to 40 cm.$^{-1}$ (see Table II). In compounds that have no (Me—P) linkage, e.g. (Me_2N)PCl_2 and (Me_2N)$(OEt)_2PO$ the $\Delta\nu_{1/2}$ value is usually about 22 to 36 cm.$^{-1}$. In two pyrophosphate compounds, [(Me_2N)(OEt)PO]$_2$O and (Me_2N)$_2$PO—O—PO$(OEt)_2$, the band is merged with other absorption bands in the region and does not appear as a discrete peak. The $\Delta\nu_{1/2}$ value for two compounds with diethylamino groups is 16 cm.$^{-1}$ and, since both compounds contain (Me—PO) groups, there appears to be no absorption contributed by the diethylamino group in the region 1320 to 1280 cm.$^{-1}$. From the results of the spectra examined it appears that absorption in the region 1320 to 1280 cm.$^{-1}$ is characteristic of dimethylamino groups in phosphonic acid esters, and it may be distinguished from absorption arising from (Me—PO) groups by the $\Delta\nu_{1/2}$ values: $\Delta\nu_{1/2}$ = 30 cm.$^{-1}$ for (Me_2N), 16 cm.$^{-1}$ for (Me—PO), and 35 cm.$^{-1}$ for both groups on the phosphorus atom. In some pyrophosphates the band may not be clearly separated from bands lying at slightly longer wavelengths (see the last two compounds of Table II).

The regions near 1190 cm.$^{-1}$ and 1064 cm.$^{-1}$ give rise to consistent absorption for all the compounds studied (see Table II). The maximum and minimum values of the 1190 cm.$^{-1}$ band are 1175 and 1192 cm.$^{-1}$, and of the 1064 cm.$^{-1}$ band, 1063 and 1070 cm.$^{-1}$ respectively. It may be noticed that all compounds give a discrete peak in the 1190 cm.$^{-1}$ region with the exception of (Et_2N)$_2$MePO with two peaks, at 1192 and 1177 cm.$^{-1}$. There appears to be no certain method of determining the assignment here, but tentatively the 1192 cm.$^{-1}$ peak was chosen as being the more intense. In two pyrophosphates the 1064 cm.$^{-1}$ peak is merged with other absorption bands.

Absorption in the region 1050–950 cm.$^{-1}$ is frequently associated with the (P—O—C) linkage, suggested to arise more specifically from the P—O—(C) bond. Examination of the spectra of three compounds without this linkage, e.g., (Me_2N)PCl_2, (Me_2N)$POCl_2$, and (Me_2N)$_2$POCl, discloses a strong absorption band in the 1000 to 980 cm.$^{-1}$ region. A similar absorption band can be found in the remainder of the compounds, the minimum and maximum values of wavelength being 998 and 978 cm.$^{-1}$. The range of absorption is

*$\Delta\nu_{\frac{1}{2}}$ *is defined as the width of the absorption band in cm.*$^{-1}$ *at half the maximum optical density. D. A. Ramsay has derived an expression (J. Am. Chem. Soc. 74: 72. 1952)*

$$A = (K/cl)\log_e[(T_0)/(T)\nu_{max}] \times \Delta\nu_{\frac{1}{2}}^{a}$$

where $K \simeq \pi/2$ *for condition of slit width much smaller than band width at half intensity,*
c is the concentration of the solution in moles per liter,
l is the absorption path length,
$\log_e[(T_0)/(T)\nu_{max}]$ *is the apparent peak intensity of the band at the frequency* ν_{max},
$\Delta\nu_{\frac{1}{2}}^{a}$ *is the apparent half intensity width of the band.*
This formula permits comparisons of integrated band intensities at different parts of the spectrum. For the comparison of bands on a quantitative basis linear wavelength absorption curves are replotted on a linear wave number scale and the area under the curve determined. For comparison of absorption bands common to a series of a molecular species, values of $\Delta\lambda_{\frac{1}{2}}$ *(microns) may be substituted for* $\Delta\nu_{\frac{1}{2}}$ *(cm.*$^{-1}$*) without serious error for bands occurring over a narrow range of frequencies.*

rather narrow and the substitution of ethyl for methyl appears to shift the absorption band out of this region.

A calculation of the absorption frequency of the (P—N) stretching vibration from Gordy's rule gives a value of 735 cm.$^{-1}$ or 13.6 μ. Corbridge and Lowe (5) have observed strong absorption near 14.3 μ (700 cm.$^{-1}$). The position of this band may be expected to be variable depending on the characteristics of the other groups associated with the phosphorus atom. The assignment of specific bands to any linkage is uncertain since the region 770 to 670 cm.$^{-1}$ is usually associated with skeletal vibrations which may be strongly influenced by comparatively small structural changes. The last column of Table II suggests absorption bands which may arise out of (P—NMe_2) vibrations. In this column the absorption bands are designated as weak (w), medium (m), or strong (s).

An examination of the position of the absorption band arising from the (P═O) bond indicates a shift toward longer wavelengths when a dimethylamino or a diethylamino group is substituted into the molecule. The shift of the (P═O) band position is shown in Table III for several compounds.

TABLE III

EFFECT OF (Me_2N) GROUPS AND (Et_2N) GROUPS ON THE POSITION OF (P═O) ABSORPTION BAND POSITION (CM.$^{-1}$)

Compound	Number of (Me_2N) substituent groups		
	0	1	2
$POCl_3$	1305	1268	1241
$(EtO)_3PO$	1277	1250	1220
$(EtO)_2PO—O—PO(OEt)_2$	1298	1268	1242 1234
$(EtO)_2MePO$	1243	1234	1205
$(EtO)_2MePO$*	1243	1238	1221

**The substituent group was* (Et_2N) *in place of* (Me_2N).

In all cases but the first, the shift of band position is greater on substitution of a second dialkylamino group into the molecule.

The effect of a substituted dimethylamino group on the magnitude of the band shift is greater than that of a diethylamino group for one set of compounds. A correlation between the substituent groups and the position of the (P═O) band has been put forward by several authors and elaborated in (1). The relationship is

$$\lambda(\mu)(P{=}O) = (39.96 - \sum x)/3.995$$

where $\lambda(\mu)$(P═O) is the expected wavelength for the phosphoryl absorption band in microns.

$\sum x$ is the sum of the "phosphoryl absorption shift constants" of the substitu-

ents attached to the phosphorus atom. Values are quoted for x for a wide range of atoms and radicals and a value of 3.0 is assigned to the (NR_2) group. Using this relationship the "phosphoryl absorption shift constants" for the dimethylamino and diethylamino groups have been calculated for the compounds in Table II. The values are 2.46±0.1 average deviation for the dimethylamino group (14 compounds), and 2.75±0.1 average deviation for the diethylamino group (2 compounds).

Absorption of radiation in the region of 3.4 μ (2940 cm.$^{-1}$) has been associated with (C—H) stretching vibrations (10, p. 195). The absorption bands observed in the region 2860 to 2780 cm.$^{-1}$ are probably attributable to (C—H) stretching in the dimethylamino group. The failure of diethylamino substituents to show absorption in this region is not clear since such absorption is present in the spectrum of diethylamine itself. Absorption in the region of 6.8 μ (1470 cm.$^{-1}$) is attributed to bending vibrations in the methyl group (6; 8; 10, p. 195). Absorption at 7.56 μ and 7.65 μ (1325 and 1308 cm.$^{-1}$) is associated with methyl symmetrical bending in Me_3PO (6). Absorption bands are observed in these two regions for compounds containing dimethylamino groups but they appear to be shifted when the substituent is diethylamino. Energy absorptions at 1190 cm.$^{-1}$ and 1064 cm.$^{-1}$ occur over rather narrow bands for a wide variety of substituents on the phosphorus atom and the vibration mode may not include the phosphorus atom itself. A calculation by Gordy's rule (9) suggests a wavelength of 8.5 μ (1178 cm.$^{-1}$) for the (C—N) stretching vibration and the bands at 8.4 μ and 9.4 μ (1190 cm.$^{-1}$ and 1064 cm.$^{-1}$) may arise out of the dialkylamino skeleton $\left(C\begin{smallmatrix}\diagup N\\ \diagdown N\end{smallmatrix}\right)$. This is supported by the fact that the wavelength varies only slightly from compound to compound, the band falls roughly in the same region as the (P)—O—C bands, and the terminal group may be ethyl or methyl without shifting the band position very much. The absorption band observed in the 1000 to 980 cm.$^{-1}$ region may involve the terminal methyl group since change of substituent from dimethylamino to diethylamino causes the band to be shifted from the region. The (P—N) stretching vibration should give rise to absorption near 13.6 μ (735 cm.$^{-1}$) according to Gordy's rule (9), but the assignment of specific bands in the 13 to 15 μ (770 to 670 cm.$^{-1}$) region to any linkage is uncertain, as the region is usually associated with skeletal vibrations, which may be strongly influenced by comparatively small structural changes.

In summary, the bands which appear to be specific for the dimethylamino group in phosphonic acid esters are 1330 to 1300 cm.$^{-1}$, 1000 to 980 cm.$^{-1}$, and possibly 2860 to 2780 cm.$^{-1}$. Other bands associated with the dimethylamino and diethylamino groups are 1470 to 1450 cm.$^{-1}$, 1190 cm.$^{-1}$, 1064 cm.$^{-1}$, and perhaps 770 to 670 cm.$^{-1}$, but in the absence of bands giving confirmation to assignments in the latter region, the added evidence is not strong. An additional bit of information may be derived from the position of the absorption band for the (P=O) bond which may be displaced toward longer wavelengths as much as 64 cm.$^{-1}$ when two substituent dimethylamino groups are put into the molecule.

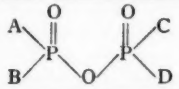
(b) The Pyrophosphate Grouping (PO—O—PO)

Interest in the group of compounds of the Schradan type and in the pyrophosphates generally has led to an investigation of the possibility of distinguishing the (PO—O—PO) linkage from the point of view of recognition in unknowns, or of testing reaction products for the formation of the linkage. Holmstedt and Larrson (11) have suggested that the (P—O—P) linkage may give rise to absorption at about 14.1 μ (710 cm.$^{-1}$) on a basis of two derivatives of pyrophosphoric acid. Bergmann, Littauer, and Pinchas (3) have observed a band at 10.3 to 10.6 μ (970 to 944 cm.$^{-1}$) which they attribute to the (P—O—P) linkage. The band is not so apparent in the *n*-propyl and isopropyl esters and it is suggested that the wavelength is shifted so that the band cannot be distinguished from the 9.8 μ (1020 cm.$^{-1}$) absorption of the (P—O—C) linkage. Corbridge and Lowe (5) compared the (P—O—P) stretching vibration with the (Si—O—Si) stretching at 9.5–9.7 μ (1052 to 1031 cm.$^{-1}$) and the (P—O—C aliphatic) at 9.3–9.9 μ (1077 to 1010 cm.$^{-1}$). They observe that (P—O—P) stretching bands near 11 μ (910 cm.$^{-1}$) appear to shift toward longer wavelengths with increasing length of the (P—O—P) chain in the inorganic pyrophosphates they studied. Absorption in pyrophosphates is usually limited to a single band near 14.2 μ (704 cm.$^{-1}$), but this may become double in triphosphates and metaphosphates. They suggest that some bands observed near 14.2 μ (704 cm.$^{-1}$) may be due to (P—O) bending in the (P—O—P) link.

Some compounds prepared in these laboratories were examined in the 950 to 910 cm.$^{-1}$ and the 715 cm.$^{-1}$ regions of the spectrum and the results are given in Table IV, together with the characteristic absorptions from compounds of a similar type prepared by other workers (7). Since these compounds are limited to substituted pyrophosphates, the column labelled "Compound"

TABLE IV

ABSORPTION BANDS CHARACTERISTIC OF THE PO—O—PO LINKAGE



A	B	C	D		Absorption bands (cm.$^{-1}$) 950–900	$\Delta\nu_{\frac{1}{2}}$*	710–670‡
EtO	Me	Me	OEt	Fig. 14	943	62	<670
OiPr	Me	Me	OiPr	Fig. 15	945	60	<670
O*sec*Bu	Me	Me	O*sec*Bu	Fig. 16	947vb	64	714vw
OEt	Et	Et	OEt	Fig. 17	922†	—	712
OiPr	Et	Et	OiPr	Fig. 18	922	58	713
O*sec*Bu	Et	Et	O*sec*Bu	Fig. 19	928	51	704vw
EtO	EtO	EtO	EtO	—	940vb	—	697w
Me_2N	Me_2N	Me_2N	Me_2N	—	925	55	670s
Me_2N	Me_2N	OEt	Me_2N	Fig. 12	926	52	668\ 690/
Me_2N	OEt	Me_2N	OEt	—	938vb	—	—
Me_2N	Me_2N	OEt	OEt	Fig. 13	940	48	710
MeO	MeO	MeO	MeO	Ref. 7	980	—	704
$(P_2O_6)_2$				Ref. 7	1010	—	764
BuO	BuO	BuO	BuO	Ref. 7	953	—	700

$\Delta\nu_{\frac{1}{2}}$ = band width in cm.$^{-1}$ at half optical density.

†Spectrum was obtained from CS_2 solution.

‡Abbreviations in this column refer to band intensity: vw, very weak; w, weak; s, strong.

gives the four substituents in the molecule. Strong absorption is observed in the 950 to 910 cm.$^{-1}$ regions in all compounds except two, viz. $(MeO)_2$-PO—O—PO $(OMe)_2$ and P_4O_{10}. With respect to the former compound, Bergmann, Littauer, and Pinchas (3) comment that the behavior of the first member of a homologous series may not be characteristic of the series. The absorption bands occur over a range of 947 cm.$^{-1}$ maximum to 922 cm.$^{-1}$ minimum. From an examination of the spectra of compounds without the (P—O—P) linkage, it appears unlikely that confusion would occur with other bands, with the exception of that from the ethyl ester group (EtO—P) which usually occurs at 962 to 944 cm.$^{-1}$ with a shoulder at 944 to 926 cm.$^{-1}$. Examination of the height and of the width at half height ($\Delta\nu_{1/2}$) indicates that the (EtO—P) band has a value of $\Delta\nu_{1/2}$ = 32 to 40 cm.$^{-1}$ in five phosphonic acid esters. The value of $\Delta\nu_{1/2}$ for the pyrophosphate derivatives varies from 48 to 64 cm.$^{-1}$ (average 56 cm.$^{-1}$) with two compounds exhibiting peaks broadened so that continuous absorption occurs over the 1000 to 910 cm.$^{-1}$ region.

Absorption in the region 715 to 670 cm.$^{-1}$ is strong and well defined in only a few cases—Fig. 13, and for the compounds $(NMe_2)_2PO—O—PO(NMe_2)_2$, $(MeO)_2PO—O—PO(OMe)_2$, P_2O_5, and $(BuO)_2PO—O—PO(OBu)_2$. The range of wavelength is considerable, and absorption bands occurring at the limit of the range of the instrument are labelled "<670". P_2O_5 exhibits a well-defined absorption at 763 cm.$^{-1}$ and this shortening may correlate with the band at 1010 cm.$^{-1}$ instead of 950 to 910 cm.$^{-1}$. In general it would not appear reliable to attribute absorption bands in the 715 to 670 cm.$^{-1}$ region to the (P—O—P) linkage but they may be confirmatory in the presence of strong broad absorption at 950 to 910 cm.$^{-1}$.

Briefly, organic esters of phosphorus-containing compounds with a linkage (P—O—P) may best be distinguished by the presence of a strong broad absorption band in the spectral region 950 to 910 cm.$^{-1}$. The ethyl ester grouping (EtO—P) is most likely to be confused, but the width of the band at half intensity ($\Delta\nu_{1/2}$) for five esters with this grouping is not greater than 40 cm.$^{-1}$, while the average value of $\Delta\nu_{1/2}$ for the pyrophosphates considered is 56 cm.$^{-1}$. An additional absorption band at 715 to 670 cm.$^{-1}$ may furnish additional evidence, but it is confirmatory only.

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THE ANALYSIS OF FLUOROCARBONS: USE OF INFRARED SPECTROPHOTOMETRY FOR THE ANALYSIS OF SMALL SAMPLES[1]

By P. B. Ayscough[2]

ABSTRACT

A method for the analysis of small quantities of mixtures of carbon tetrafluoride, hexafluoroethane, and fluoroform based on measurement of the intensity of infrared absorption bands is described. The relation between pressure and optical density for the main absorption bands of each gas was determined and used to calculate the composition of synthetic mixtures. These results were compared with those obtained by mass spectrometric analysis.

INTRODUCTION

Analytical procedures for handling small quantities of fluorocarbons are, as yet, largely undeveloped, though the physical and chemical properties of these compounds are well known. In order to make possible an examination of the reactions of trifluoromethyl radicals it was necessary to find an accurate and rapid method for the analysis of carbon tetrafluoride, hexafluoroethane, and fluoroform in quantities of 10 micromoles or less.

A summary of the various methods for analyzing fluorine compounds is to be found in Volume II of *Fluorine Chemistry* edited by J. H. Simons (Academic Press Inc., 1954). Procedures based on differences in vapor pressure or vapor density were shown to be of little value for analyzing these mixtures, and the chemical methods, such as pyrolysis over white-hot silica, combustion in moist hydrogen, and sodium fusion, give only values for the average carbon and fluorine content. Conventional oxidation methods cannot be used because of the high resistance to oxidation of fluorocarbons.

Mass-spectrometric analysis is one possible method since the mass spectra of most of the simple fluorine-containing compounds are known (2). However, a characteristic feature of the mass spectra of fluorocarbons is the almost complete absence of parent peaks and, for the straight-chain fluorocarbons, the preponderance of the mass 69 peak (CF_3^+). This means that the analysis depends on the magnitude of the relatively minor peaks which are frequently common to a large number of fluorocarbons. Carbon tetrafluoride is particularly difficult to estimate in this way because all of its peaks are shared by other fluorocarbons and it must be estimated from the residual 69 (CF_3^+) and 50 (CF_2^+) peaks.

Nevertheless, the method has been used successfully during the course of this work for the estimation of mixtures of carbon tetrafluoride, hexafluoroethane, and fluoroform in the presence of carbon monoxide and carbon dioxide. The results of a few analyses of test samples will be presented in a later section.

An examination of the infrared absorption spectra of carbon tetrafluoride,

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[2]*National Research Council Postdoctorate Fellow, 1953–1955.*

hexafluoroethane, and fluoroform showed the presence of a number of strong well-defined bands in the region 1000–1500 cm.$^{-1}$ which might be used for analysis. It was shown experimentally that with the exception of carbon tetrafluoride these compounds obey Beer's law in this region and that for samples of 5–10 micromoles an accuracy of $\pm 5\%$ can be obtained. The results of experiments designed to determine the relation between intensity and pressure for these compounds are presented in this paper, together with the analysis of some test samples.

Although the spectra of higher fluorocarbons are more complex it is reasonable to suppose that this method can be extended to estimate mixtures of other fluorocarbons in the same way as it is applied to the estimation of complex mixtures of hydrocarbons.

EXPERIMENTAL

Apparatus

Optical density measurements were made using a Perkin-Elmer Model 21 double beam recording spectrophotometer with a sodium chloride prism. The optical cell used in conjunction with this instrument was made of pyrex with polished sodium chloride windows fixed with Benolite cement. The cell had a volume of 155 ml. and was fitted with a stopcock and ground glass joint for admitting the samples of gas. Silicone grease was used on all joints to reduce absorption of the gas. No absorption of gas on the walls of the vessel was observed. The pressure of gas in the optical cell was calculated by measuring the pressure of the sample in a calibrated gas burette and then allowing the gas to expand into the cell. A pressure of 1 mm. in the cell is given by about 8 micromoles of gas.

Materials

Samples of carbon tetrafluoride, hexafluoroethane, and fluoroform of unspecified purity were supplied by E.I. du Pont de Nemours and Co. Inc. to whom we are greatly indebted. The gases were purified by bulb to bulb distillation in the vacuum system, a small middle fraction being taken in each case for calibration purposes. The mass spectra and infrared absorption spectra of these samples were identical with those published previously (1, 2, 5–8) and they were therefore assumed to be pure.

RESULTS AND DISCUSSION

For the purpose of analysis the important spectral region is from 1000 to 1500 cm.$^{-1}$. Since the spectra have already been published, they will not be reproduced here. The only strong line in the carbon tetrafluoride spectrum is that at 1284 cm.$^{-1}$, which is well separated from the strongest absorption bands of hexafluoroethane (1253 cm.$^{-1}$) and fluoroform (1152 cm.$^{-1}$), so that it is possible to use the intensity of these bands as a measure of the partial pressure of each gas in a mixture. Weaker bands in the hexafluoroethane spectrum (1117 and 1123 cm.$^{-1}$) and in the fluoroform spectrum (1380 cm.$^{-1}$) might be used under conditions in which the other bands are too intense.

Measurements of peak optical density were made for each band, using the pure gases, and although some deviation from Beer's law is observed in the

case of carbon tetrafluoride and fluoroform this is not serious enough to affect the validity of the method. All measurements were made with comparable slit widths with no matching cell in the control beam of the spectrophotometer. The base lines were obtained with the cell evacuated and all measurements of optical density were made from these reference lines. Tables I–III and Figs. 1–3 indicate the results of these experiments.

TABLE I

OPTICAL DENSITY OF CARBON TETRAFLUORIDE

Sample	Pressure (mm.)	Optical density, 1284 cm.$^{-1}$
A1	0.84	0.80
A2	0.646	0.75
A3	0.44	0.545
A4	0.24	0.344
A5	0.12	0.20
A6	0.95	0.92
A7	0.655	0.70
A8	0.46	0.52
A9	0.074	0.115

TABLE II

OPTICAL DENSITY OF HEXAFLUOROETHANE

Sample	Pressure (mm.)	Optical density		
		1253 cm.$^{-1}$	1123 cm.$^{-1}$	1117 cm.$^{-1}$
B1	0.470	0.690	0.130	0.200
B2	0.251	0.375	0.068	0.105
B3	0.157	0.215	0.042	0.054
B4	0.091	0.128	0.021	0.032
B5	0.652	1.032	0.215	0.302
B6	0.439	0.652	0.127	0.180
B7	0.273	0.402	0.077	0.109
B8	0.187	0.258	0.050	0.070
B9	0.129	0.173	0.033	0.047
B10	0.930		0.269	0.389

TABLE III

OPTICAL DENSITY OF FLUOROFORM

Sample	Pressure (mm.)	Optical density	
		1380 cm.$^{-1}$	1152 cm.$^{-1}$
C1	1.14	0.092	0.625
C2	0.695	0.064	0.400
C3	0.223	0.020	0.165
C4	0.152		0.105
C5	1.42	0.110	0.750
C6	0.970	0.073	0.520
C7	0.646	0.045	0.348
C8	0.385		0.236
C9	0.213		0.147
C10	0.643	0.051	0.363
C11	0.729	0.055	0.405
C12	1.73	0.123	0.80
C13	1.06	0.083	0.583

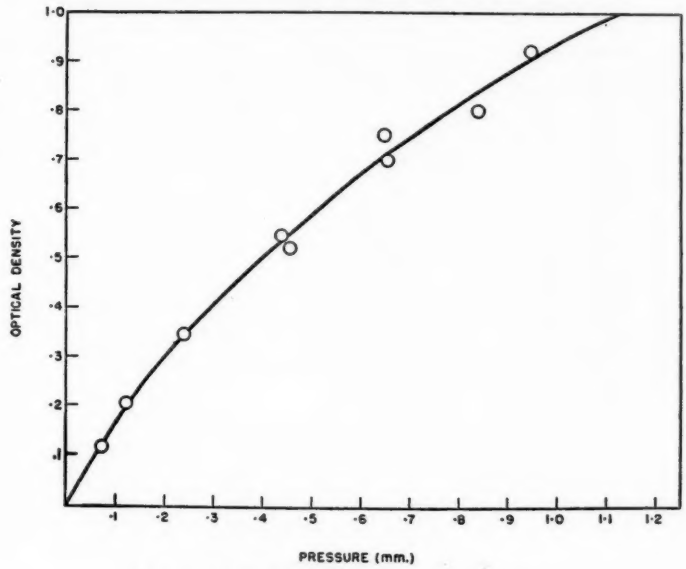

FIG. 1. Optical density of carbon tetrafluoride.

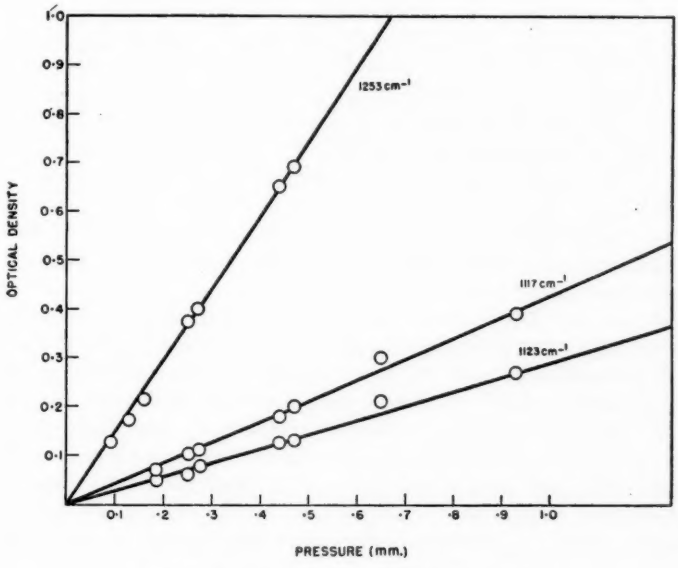

FIG. 2. Optical density of hexafluoroethane.

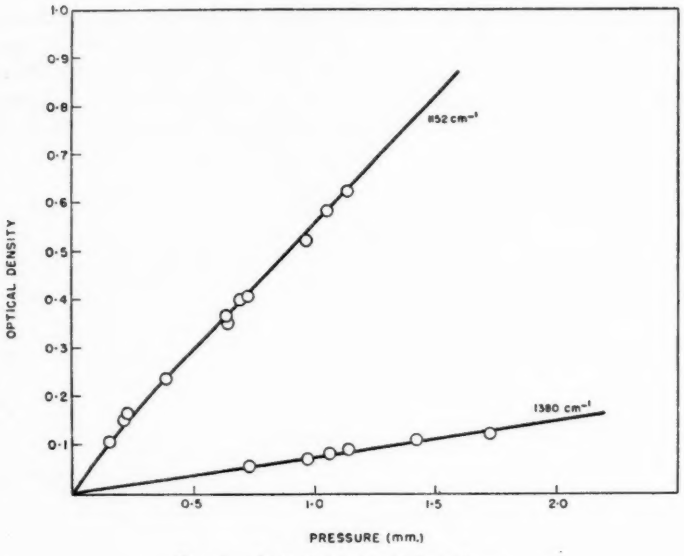

FIG. 3. Optical density of fluoroform.

Spectra of Mixtures

In order to check the accuracy of analyses using these absorption bands a number of mixtures were prepared. The absorption spectrum of each was observed under the same conditions as were used for the samples of pure materials, and from the peak heights the partial pressure of each was calculated and compared with the true values. There is very little overlap of absorption bands and it is necessary to apply a correction only in the case of the 1152 cm.$^{-1}$ band of fluoroform in the presence of hexafluoroethane. This correction becomes significant when C_2F_6/CF_3H is of the order of 5:1 or greater. The results of analyses of these test mixtures are shown in Table IV. It will be seen that there is good agreement between the values obtained in this way and those obtained by synthesis. In some cases the mixtures were also analyzed mass spectrometrically. The results of these analyses are added to the table for comparison.

Since the time this method of analysis was established, it has been used on numerous occasions in connection with a series of investigations of the reactions of trifluoromethyl radicals (3, 4). The mixtures have consisted, in general, of hexafluoroethane and fluoroform in the presence of small amounts of methane, ethane, and hexafluoroacetone. Small quantities of impurities such as these are of no importance since they do not absorb in the same region as the fluorocarbons. The only serious limitation so far observed is that resulting from pressure broadening of the fluoroform band in the presence of large amounts of hydrogen-containing material. Abnormally high results are obtained in this

TABLE IV

OPTICAL DENSITY OF MIXTURES

Sample	Volume (ml.) by synthesis			Volume (ml.) by infrared absn.			Volume (ml.) by mass spectrometer		
	CF_4	C_2F_6	CF_3H	CF_4	C_2F_6	CF_3H	CF_4	C_2F_6	CF_3H
M1	—	0.505	0.058	—	0.488	0.062	—	0.485	0.060
M2	—	0.335	0.323	—	0.344	0.320	—	0.331	0.316
M3	—	0.252	0.240	—	0.266	0.230	—	0.266	0.235
M4	—	0.146	0.249	—	0.160	0.251	—		
M5	—	0.114	0.528	—	0.120	0.510	—		
M6	—	0.040	0.657	—	0.042	0.667	—		
M7	0.030	0.183	—	0.034	0.165	—	0.034	0.170	—
M8	0.062	0.143	—	0.071	0.137	—	0.066	0.133	—
M9	0.156	0.033	—	0.152	0.035	—	0.147	0.037	—
M10	0.061	0.142	0.166	0.072	0.134	0.166	0.065	0.145	0.172
M11	0.160	0.088	0.098	0.162	0.087	0.099	0.153	0.085	0.096
M12	0.046	0.074	0.175	0.055	0.075	0.169	0.047	0.075	0.167
M13				—	0.102	0.025	—	0.104	0.026
M14				—	0.072	0.038	—	0.069	0.039
M15				—	0.096	0.044	—	0.093	0.045

way, and to overcome this difficulty new calibration curves have to be plotted for each total pressure. This is tedious, but under these conditions the mass-spectrometric method was equally unsatisfactory.

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SOME MEASUREMENTS ON THE IRON (111) – THIOCYANATE SYSTEM IN AQUEOUS SOLUTION[1]

By M. W. Lister and D. E. Rivington

ABSTRACT

A spectrophotometric study of the ferric thiocyanate system is reported. The temperature and ionic strength of the solutions were carefully controlled, and suitable precautions were taken against fading. From the data, values of the equilibrium constant for the formation of $FeSCN^{++}$ from simple ions, and of the extinction coefficients of $FeSCN^{++}$ were obtained for different temperatures and ionic strengths, with results that differed somewhat from earlier values. The effect of varying acidity was also investigated. From more concentrated solutions, values for the equilibrium constants for the formation of $Fe(SCN)_2^+$ and $Fe(SCN)_3$ were obtained; it was not necessary to postulate higher complexes.

Many investigations have been made of aqueous solutions containing ferric and thiocyanate ions, starting with the original work of Gladstone (9) just over a hundred years ago. However it was only comparatively recently that Moeller (15) and, independently, Bent and French (3) showed that in dilute solution the color was due to the ion $FeSCN^{++}$. This put a new interpretation on the measurements made on this system, and as a result considerable further work was carried out (e.g. 6, 7, 10, 12, 18). This work mostly made use of the red color as a means of investigation, but some work was also done using solvent extraction (14), or electrochemical methods (15).

The present work was begun with the intention of using the red color of $FeSCN^{++}$ as a method of determining the concentration of free ferric ions in solution, and hence of evaluating the equilibrium constants of the dissociation of other complexes formed by ferric ions with other anions. This type of investigation was made by Lanford and Kiehl (13) for the ferric phosphate system. Although earlier work covered a fair range of conditions, it became apparent that in order to have an adequate picture of the system before adding other ions, it would be desirable to extend these measurements to cover systematically a range of temperature, hydrogen ion concentration, and ionic strength. These factors were therefore carefully controlled; temperature control, for instance, was provided by using absorption cells completely immersed in a thermostatted tank. The fading which occurs in these solutions had also to be taken into account, and it is believed conditions were found such that fading was negligible. It was also necessary to take into account the possible formation of the higher complexes, $Fe(SCN)_2^+$ and $Fe(SCN)_3$; and it was in fact possible to make a moderately accurate estimate of the extent to which these species were present, and so to determine their dissociation constants. In interpreting the experimental data, it is not necessary to assume that the extinction coefficients at various wave lengths were constant over a range of conditions; so that these experiments enable one to find the effect of temperature and ionic strength on the extinction coefficients. The effect of hydrogen ion concentration was

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Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario.

also examined, especially as regards the possible formation of the ion $FeOH^{++}$ and of undissociated thiocyanic acid. It is hoped therefore that the present work provides a reasonably critical and detailed account of the ferric thiocyanate system, and one which can be used in measuring the dissociation constants of other ferric complexes.

APPARATUS AND MATERIALS

Spectrophotometer

All optical measurements were made with the Beckmann Model DU Spectrophotometer. For normal slit widths in the wave length region used in this work (4000–5000 Å) this instrument uses a band of wave lengths about 10 Å wide (8). As earlier workers do not entirely agree about the position of the maximum absorption of $FeSCN^{++}$, the wave length scale was calibrated using the hydrogen and mercury spectra, and was found to agree within 3 Å (i.e. within experimental error) at the wave lengths of the well-established lines of these elements. The absorption cuvettes used were made of silica, and were of conventional design. The light path was usually 1 cm. and was calibrated to $\pm 0.1\%$. For shorter light paths silica inserts, 0.9 cm. thick, were put in to reduce the light path to 1 mm.

The chief modification that was made on the spectrophotometer was the introduction of thermostatting for the absorption cells. The cells were placed directly in the thermostatting water in a bakelite box fitted with silica windows. The box was light-tight except for the windows. The light beam passed through the water, which was circulated by a pump from a larger reservoir. This reservoir, which contained the usual constant temperature devices, was made of stainless steel, as were the attached pipes and valves, and was filled with distilled water to minimize absorption by impurities in the water. The flow rate through the box was about 1 liter/min., and the temperature could be easily maintained to 0.1°C.

Ferric Perchlorate

The G. Frederick Smith product was used without further purification. A stock solution of about 0.5 *M* was prepared and then analyzed for iron by ammonia precipitation, ignition, and weighing as Fe_2O_3. No check for free acid was made since a high concentration of perchloric acid was added to the mixtures in all the runs.

Perchloric Acid

The 72% reagent grade product supplied by G. Frederick Smith was diluted to give a 1 *M* stock solution which was standardized volumetrically.

Sodium Perchlorate

A 5 *M* stock solution was prepared from the G. Frederick Smith reagent grade sodium perchlorate by dissolving it in water and filtering. This solution gave no test for chloride. The concentration was determined by evaporating a known volume of solution to dryness, heating to 160°C. to break up the mono-

hydrate, and weighing the residue. A check showed that this method gave the same result as prolonged heating at 300°C. This solution produced the same results as one prepared by neutralizing a known amount of perchloric acid with reagent sodium hydroxide, and diluting to the required volume.

Sodium Thiocyanate

A 1 *M* stock solution was prepared from Baker's Analyzed product, and standardized against silver nitrate with ferric alum as indicator. Solutions of thiocyanate slowly decompose on standing to produce a flaky white precipitate. It was found that this trouble could be largely avoided by keeping the solutions in black storage bulbs in an atmosphere of nitrogen. When the solution was needed, it was driven out by a stream of nitrogen.

Benzyl Alcohol

Merck reagent grade benzyl alcohol was used, and was stored in an atmosphere of nitrogen.

Standard Ferric Solutions

It was desirable to prepare a ferric solution with its concentration accurately known in order to have an independent check on the various batches of ferric perchlorate that were prepared. For this purpose the usual iron standards such as ferrous ammonium sulphate were of no value since the sulphate interferes strongly with the ferric thiocyanate color. Consequently a length of clean iron wire was weighed to 0.1%, and heated with the required amount of perchloric acid and a small amount of ammonium nitrate to oxidize ferrous to ferric ions. After cooling, the product was diluted to a known volume and used to produce a ferric thiocyanate solution in the same way as other ferric perchlorate solutions. The intensity of the color was compared with that from the latter solutions, whose concentration could thereby be checked. The results agreed within experimental error with those obtained by precipitating ferric hydroxide.

EXPERIMENTAL

Stock solutions of ferric perchlorate, sodium thiocyanate, perchloric acid, and sodium perchlorate were made up of known strength. Mixtures of known amounts of these were made, and diluted to a known volume, and the color was measured by the spectrophotometer. The color was always measured against a reagent blank which did not contain thiocyanate. In runs where the iron concentration was low this was also omitted in the reagent blank, but at higher concentrations it was included.

It was discovered early in this part of the investigation that solutions with the ferric concentration around 0.1 *M* and thiocyanate around 10^{-4} *M* were subject to serious fading. This was apparently not the same fading reaction that interfered with the colorimetric determination of iron as reported, for example, by Peters, MacMasters, and French (17), and that was later the subject of a kinetic investigation by Betts and Dainton (4), since this latter reaction occurred in solutions of very high thiocyanate concentration and very

low ferric concentration—just the reverse of the present case. The explanation of this fading reaction that was arrived at was that the thiocyanate is oxidized by dissolved oxygen, probably with ferric ion acting as a carrier. This is not an unfamiliar role to assign to ferric and other transition element ions (see, for example, Ref. 1). Jeu and Alyea (11) give a list of substances in order of their ability to inhibit the oxidation of sulphite. From this list benzyl alcohol was selected as a substance with good inhibitory properties, but still not likely to form complexes with ferric ions. In both respects this proved to be a happy choice. The ability of benzyl alcohol to inhibit fading was observed in many solutions, even at 45°C. Qualitatively, an inhibited solution stored in a glass-stoppered volumetric flask was still decidedly red after 18 months, whereas a companion solution with no inhibitor faded completely in about 10 days.

In order to check the possible interference of benzyl alcohol in the color formation, a series of solutions were set up in which the ferric and thiocyanate concentrations were maintained constant throughout and increasing amounts of benzyl alcohol were added; the optical densities of the solutions were read. As seen from the results given in Table I the benzyl alcohol does not interfere to any significant extent.

TABLE I

EFFECT OF BENZYL ALCOHOL

Iron and thiocyanate: 0.001 *M*; (H^+) = 0.2 *M*; 25°C.; volume: 25 ml.

Drops of benzyl alcohol	0	1	2	4	8
Optical density (460 mμ)	0.496	0.498	0.496	0.493	0.499

In measuring the optical density on the spectrophotometer, no reading was accepted if it showed more than 0.001 change in density after the 'dark current' and blank absorption were checked. At least two, and usually three, readings were taken for each wave length; the density reading was first taken for each wave length used, and then the whole series repeated. It was found that about six drops of benzyl alcohol in 50 ml. of solution were sufficient to reduce fading so that this degree of reproducibility could be obtained.

The results obtained are given in Tables II–VI, and the interpretation of these results will be discussed in the next section. However there is one general comment that should be made first. The ionic strength of 1.2 which was used in many runs may seem unduly high. It was originally chosen to allow, firstly, a sufficient concentration of acid to be present to suppress the formation of $FeOH^{++}$; and, secondly, to allow for a fairly high concentration of ferric perchlorate in some runs. As it turned out this ionic strength was higher than was needed in most runs, but it was thought to be advisable to keep it constant throughout the whole series. In Table III results are given which show the effect of altering the ionic strength.

In all of these tables the optical densities have been reduced to the density per centimeter by dividing by the calibrated cell width.

TABLE II

VALUES OF OPTICAL DENSITY FOR 1 CM. LIGHT PATH

All measurements in this table at 25°C., 1.2 ionic strength, and $(H^+) = 0.2\ M$

Series	(SCN^-), M	(Fe^{+3}), M	Wave length, mμ										
			400	420	430	440	450	460	470	480	490	500	520
1	.000125	.1667	.268	.411	.477	.523	.551	.560	.543	.511	.468	.416	.303
		.125	.266	.407	.469	.513	.543	.551	.539	.503	.461	.412	.300
		.100	.259	.399	.461	.508	.536	.540	.528	.498	.452	.401	.295
		.0833	.259	.395	.458	.500	.530	.537	.521	.491	.449	.396	.289
		.0667	.247	.385	.448	.489	.513	.522	.509	.479	.439	.387	.283
		.0500	.240	.370	.429	.471	.498	.502	.489	.461	.422	.373	.274
		.0333	.225	.347	.401	.441	.466	.471	.460	.436	.399	.352	.257
		.0200	.200	.309	.356	.391	.414	.420	.410	.385	.352	.313	.229
		.0100	.156	.239	.279	.305	.324	.325	.320	.301	.275	.243	.177
2	.000100	.1667	.207	.331	.385	.426	.448	.453	.440	.418	.379	.335	.244
		.125	.212	.329	.381	.420	.441	.449	.438	.412	.374	.331	.243
		.100	.209	.324	.376	.414	.437	.442	.430	.408	.370	.328	.240
		.0833	.205	.319	.370	.409	.430	.437	.423	.401	.363	.322	.237
		.0667	.200	.311	.360	.396	.419	.422	.412	.388	.355	.314	.229
		.0500	.192	.301	.350	.381	.404	.410	.400	.376	.344	.305	.225
3	.00025	.000985	—	.096	—	.121	.128	.130	.126	.119	.110	.097	—
	.00050		—	.189	—	.238	.250	.254	.247	.234	.214	.189	—
	.00075		—	.275	—	.350	.368	.373	.363	.343	.314	.279	—
	.00100		—	.358	—	.458	.479	.487	.470	.448	.411	.364	—
	.00125		—	.441	—	.559	.589	.598	.587	.555	.505	.449	—
	.00150		—	.510	—	.650	.683	.696	.681	.642	.590	.525	—
	.00175		—	.588	—	.752	.792	.810	.790	.743	.681	.604	—
	.00200		—	.661	—	.846	.889	.904	.879	.834	.762	.681	—
	.00225		—	.797	—	1.012	1.071	1.089	1.067	1.008	.929	.829	—
	.00250		—	.927	—	1.171	1.244	1.265	1.232	1.172	1.085	.966	—
4	.00040	.00197	—	.257	—	.333	.349	.356	.346	.327	—	.263	—
	.00060		—	.387	—	.495	.525	.534	.521	.489	—	.394	—
	.00080		—	.520	—	.663	.701	.709	.693	.653	—	.531	—
	.00100		—	.646	—	.830	.870	.886	.863	.814	—	.655	—
5	.00020	.00298	—	.187	—	.238	.250	.253	.246	.233	—	.188	—
	.00040		—	.364	—	.466	.487	.495	.484	.455	—	.367	—
	.00060		—	.545	—	.699	.733	.743	.725	.653	—	.582	—
	.00080		—	.722	—	.913	.967	.974	.950	.903	—	.727	—
6	.00015	.0167	.255	.394	.452	.498	.521	.527	.510	.480	.436	.384	.279
		.0150	.251	.385	.443	.487	.509	.514	.500	.469	.428	.375	.274
		.0125	.236	.365	.420	.460	.481	.489	.471	.443	.406	.357	.260
		.0100	.220	.338	.390	.428	.449	.451	.440	.411	.375	.331	.240
		.0075	.195	.300	.348	.381	.400	.405	.390	.367	.334	.296	.214
		.0050	.160	.249	.286	.315	.329	.331	.321	.304	.275	.245	.176
		.0025	.105	.163	.189	.205	.215	.217	.213	.199	.181	.159	.116

TABLE III

EFFECT OF IONIC STRENGTH

Optical densities at 1 cm. light path; 25°C.

Series	(SCN^-), M	(Fe^{+3}), M	Wave length, mμ					Ionic strength
			440	450	460	470	480	
1	.000250	.000985	.125	.130	.134	.128	.120	0.8
	.000500		.243	.255	.258	.251	.235	
	.000750		.355	.376	.380	.368	.349	
	.00100		.460	.486	.494	.479	.456	
	.001243		.566	.595	.604	.588	.554	
	.001492		.665	.703	.712	.694	.653	
	.001742		.766	.811	.823	.795	.752	
	.001989		.855	.905	.913	.891	.848	
	.002235		.950	.992	1.010	.986	.931	
	.002481		1.037	1.091	1.109	1.085	1.026	
2	.00025	.000995	.136	.141	.144	.140	.130	0.5
	.00050		.267	.278	.281	.274	.255	
	.00075		.390	.409	.412	.400	.379	
	.00100		.498	.528	.534	.517	.488	
	.001243		.620	.649	.657	.640	.607	
	.001492		.729	.764	.774	.752	.713	
	.001742		.842	.879	.889	.867	.816	
	.001989		.933	.976	.991	.966	.911	
	.002235		1.030	1.085	1.091	1.068	1.008	
	.002481		1.127	1.184	1.192	1.168	1.107	
3	.00025	.000990	.151	.157	.159	.155	.145	0.3
	.00050		.295	.310	.314	.304	.287	
	.00075		.433	.453	.458	.447	.420	
	.00100		.558	.587	.595	.579	.547	
	.001243		.685	.719	.725	.703	.663	
	.001492		.808	.848	.853	.832	.781	
	.001742		.915	.966	.972	.949	.895	
	.001989		1.032	1.085	1.093	1.068	1.008	
	.002250		1.133	1.190	1.204	1.182	1.113	
	.002500		1.230	1.289	1.309	1.283	1.210	
4	.00025	.000990	.169	.175	.179	.172	.164	0.2
	.00050		.329	.345	.350	.340	.319	
	.00075		.486	.508	.512	.499	.469	
	.00100		.616	.648	.656	.637	.604	
	.00125		.760	.798	.810	.784	.742	
	.00150		.893	.935	.948	.927	.875	
	.00175		1.014	1.069	1.083	1.053	.991	
	.00200		1.131	1.188	1.210	1.175	1.113	
	.00225		1.251	1.319	1.331	1.291	1.230	
	.00250		1.360	1.429	1.445	1.414	1.333	

TABLE IV

EFFECT OF TEMPERATURE

Optical densities at 1 cm. light path; ionic strength 1.2

Series	Temp., °C.	(SCN^-), *M*	(Fe^{+3}), *M*	Wave length, mμ						
				420	440	450	460	470	480	500
1	5.0	.000100	.100	.340	.431	.450	.452	.439	.410	.324
			.0833	.335	.427	.443	.448	.432	.407	.319
			.0667	.329	.419	.439	.440	.428	.399	.315
			.0500	.327	.414	.432	.437	.421	.393	.312
			.0333	.311	.390	.410	.411	.398	.371	.295
			.0200	.281	.352	.368	.370	.360	.333	.265
2	45.0	.000100	.1250	.304	.394	.420	.429	.421	.401	.334
			.0833	.296	.382	.408	.418	.410	.391	.325
			.0667	.289	.371	.396	.407	.400	.381	.318
			.0500	.279	.359	.381	.391	.385	.366	.306
			.0333	.259	.335	.356	.365	.359	.343	.285
3	5.3	.00040	.000985	.185	.233	.244	.248	.238	.224	.175
	5.6	.00060		.267	.335	.352	.354	.342	.318	.254
	5.4	.00080		.348	.438	.457	.459	.448	.419	.332
	5.4	.00100		.429	.541	.567	.569	.555	.517	.410
	5.2	.00020	.00197	.163	.205	.214	.215	.209	.195	.154
	5.2	.00040		.322	.407	.427	.428	.417	.387	.307
	5.2	.00060		.473	.598	.626	.631	.608	.565	.450
	5.2	.00080		.627	.782	.828	.831	.808	.745	.594
4	10.3	.00080	.000985	.329	.418	.437	.438	.428	.400	.319
		.00100		.409	.517	.544	.547	.528	.496	.397
		.00060	.00197	.445	.562	.592	.596	.580	.541	.430
		.00080		.592	.745	.784	.790	.768	.719	.572
5	14.9	.00040	.000985	.163	.205	.215	.218	.211	.199	.159
	14.8	.00060		.243	.304	.319	.323	.313	.293	.235
	15.0	.00080		.317	.401	.420	.427	.415	.387	.312
	15.1	.00100		.388	.490	.516	.522	.506	.476	.382
	14.9	.00040	.00197	.285	.366	.384	.387	.375	.350	.279
	14.9	.00060		.426	.543	.570	.575	.555	.525	.418
	14.9	.00080		.565	.713	.750	.756	.735	.694	.553
6	20.0	.00080	.000985	.302	.383	.404	.408	.397	.373	.302
	20.1	.00100		.372	.469	.496	.499	.487	.458	.372
	20.1	.00060	.00197	.406	.517	.543	.549	.537	.504	.406
	20.1	.00080		537	.683	.723	.732	.713	.671	.537
7	30.0	.00080	.000985	.279	.355	.376	.382	.373	.553	.288
		.00100		.343	.438	.463	.468	.458	.437	.356
		.00080	.00197	.495	.636	.674	.683	.671	.636	.517
8	35.0	.00040	.000985	.136	.175	.185	.189	.184	.174	.143
		.00060		.203	.260	.274	.279	.273	.259	.213
		.00080		.269	.343	.364	.370	.363	.343	.282
		.00100		.334	.427	.448	.457	.447	.427	.348
		.00060	.00197	.365	.467	.495	.504	.495	.467	.385
		.00080		.485	.624	.661	.670	.656	.626	.514
		.00100		.603	.772	.816	.834	.814	.774	.636
9	40.0	.00080	.000985	.263	.335	.355	.363	.357	.339	.280
		.00100		.323	.416	.438	.447	.439	.419	.347
		.00060	.00197	.349	.453	.479	.489	.483	.457	.378
		.00080		.465	.602	.636	.651	.641	.611	.504
10	45.2	.00040	.000985	.129	.168	.179	.184	.180	.171	.143
		.00060		.193	.248	.263	.269	.265	.253	.209
		.00080		.255	.327	.347	.354	.349	.332	.277
		.00100		.314	.407	.429	.438	.434	.415	.343

TABLE V

DATA FOR INVESTIGATION OF HIGHER COMPLEXES

Optical densities at 1 cm. light path; $(Fe^{+3}) = 0.001\ M$; $(H^+) = 0.2\ M$; ionic strength = 1.2, for all measurements; 25°C.

(SCN^-), M	Wave length, mμ							
	420	440	450	460	470	480	490	500
.00025	.096	.121	.128	.130	.126	.119	.110	.097
.00050	.189	.238	.250	.254	.247	.234	.214	.189
.00075	.275	.350	.368	.373	.363	.343	.314	.279
.0010	.358	.458	.479	.487	.470	.448	.411	.364
.00125	.441	.559	.589	.598	.587	.555	.505	.449
.0015	.510	.650	.683	.696	.681	.642	.590	.525
.00175	.588	.752	.792	.810	.790	.743	.681	.604
.0020	.661	.846	.889	.904	.879	.834	.762	.681
.00225	.735	.931	.988	1.006	.978	.927	.851	.760
.0025	.797	1.012	1.071	1.089	1.067	1.008	.929	.829
.0030	.927	1.171	1.244	1.265	1.232	1.172	1.085	.966
.0035	1.08	1.36	1.44	1.45	1.44	1.36	1.25	1.11
.0040	1.20	1.51	1.595	1.62	1.59	1.51	1.395	1.245
.0045	1.305	1.655	1.75	1.765	1.75	1.66	1.53	1.38
.0050	1.445	1.84	1.93	1.955	1.94	1.84	1.70	1.51
.0060	1.64	2.06	2.20	2.245	2.20	2.095	1.945	1.74
.0070	1.81	2.26	2.40	2.45	2.41	2.30	2.14	1.93
.0080	1.955	2.46	2.60	2.66	2.62	2.51	2.35	2.105
.0090	2.09	2.64	2.79	2.85	2.81	2.70	2.515	2.29
.0100	2.25	2.85	3.00	3.095	3.05	2.945	2.75	2.49
.0120	2.46	3.11	3.29	3.36	3.35	3.24	3.04	2.76
.0140	2.645	3.345	3.52	3.63	3.605	3.495	3.28	3.00
.0160	2.82	3.56	3.76	3.89	3.87	3.75	3.54	3.25
.0180	2.99	3.77	4.02	4.17	4.175	4.07	3.83	3.51
.0200	3.15	3.96	4.21	4.32	4.32	4.21	4.005	3.69
.0250	3.40	4.305	4.57	4.70	4.72	4.635	4.42	4.11
.0300	3.67	4.61	4.91	5.07	5.11	5.00	4.80	4.49
.0400	4.005	5.00	5.37	5.58	5.63	5.58	5.38	5.00
.0500	4.27	5.33	5.70	5.98	6.04	6.00	5.87	5.50
.0600	4.49	5.61	5.99	6.305	6.42	6.40	6.21	5.90
.0700	4.61	5.81	6.27	6.56	6.73	6.73	6.58	6.23
.0800	4.70	5.93	6.40	6.78	6.92	6.98	6.80	6.47
.0900	4.90	6.17	6.65	7.00	7.21	7.24	7.09	6.74
.1000	5.02	6.31	6.81	7.20	7.42	7.46	7.30	6.97
.1500	5.43	6.81	7.42	7.90	8.23	8.30	8.12	7.84
.2000	5.66	7.19	7.87	8.43	8.78	8.88	8.72	8.37
.2500	5.79	7.48	8.12	8.67	9.04	9.18	8.99	8.63

TABLE VI

EFFECT OF HYDROGEN ION CONCENTRATION

Optical densities at 1 cm. light path; $(Fe^{+3}) = 0.001\ M$; $(SCN^-) = 0.001\ M$; ionic strength = 1.2; 25°C.

(H^+), M	Wave length, mμ				
	440	450	460	470	480
0.500	.459	.482	.490	.479	.453
0.200	.459	.481	.489	.478	.451
0.150	.458	.480	.488	.474	.450
0.100	.448	.470	.477	.466	.442
0.075	.448	.469	.476	.466	.440
0.050	.443	.466	.472	.460	.435
0.025	.431	.452	.460	.450	.423
0.0175	.427	.448	.454	.442	.419
0.0100	.408	.428	.432	.422	.399
0.0075	.386	.408	.410	.400	.378
0.0050	.367	.384	.388	.376	.357
0.0025	.328	.344	.347	.337	.318
0.00175	.300	.315	.317	.308	.289
0.0010	.260	.271	.273	.265	.249

DISCUSSION OF RESULTS

In the treatment of data of this sort it is usual to assume that a reaction such as

$$Fe^{+3}+SCN^{-} \rightleftharpoons FeSCN^{++}$$

conforms to a Mass Law expression

$$a_{FeSCN^{++}}/a_{Fe^{+3}}.a_{SCN^{-}} = K_0$$

where $a_{Fe^{+3}}$, etc., are the activities of the various substances. Indeed this is little more than a matter of definition, the real assumptions being that the activity coefficients are dependent only on temperature and ionic strength, and that they approach unity as the ionic strength approaches zero. In practice it is usually simpler to evaluate an apparent constant, K, defined by the concentrations; and then to observe the variation of K with ionic strength. K is in fact the equilibrium constant at a certain ionic strength and temperature, and most of the constants derived below are of this sort.

In evaluating K from optical densities, it is always assumed that the densities obey Beer's Law: $D = \epsilon.c$; where D is the density, ϵ is the extinction coefficient, and c is the concentration of the colored substance. ϵ is, of course, dependent on the wave length, and to a lesser extent on the ionic strength and temperature; but we assume that it is independent of c, if c is fairly small. In the present work the concentration of the colored ion was 10^{-3} M or less, so that it can be reasonably assumed that the colored ions are sufficiently far apart not to affect each other's extinction coefficient. The results in Tables II–V enable the effect of temperature and ionic strength on the extinction coefficient to be determined.

To obtain values of K and ϵ from optical densities somewhat similar methods are available, devised by Rabinowitch and Stockmayer (19), and by Frank and Oswalt (7). The relation of these two methods can be seen from the following, which provides a simpler derivation of Frank and Oswalt's equation. If a is the total ferric concentration (present either as Fe^{+3} or $FeSCN^{++}$), b is the total thiocyanate concentration, and x that of the complex, $FeSCN^{++}$, then:

$$x/[(a-x)(b-x)] = K.$$

As $x = D/\epsilon$, we can substitute and rearrange to get

$$ab/D = (a+b)/\epsilon + 1/\epsilon K - D/\epsilon^2.$$

If we ignore D/ϵ^2 as small compared to the other terms, we get Frank and Oswalt's equation. By plotting ab/D against $a+b$, ϵ and K can be evaluated. Rabinowitch and Stockmayer, in effect, take Frank and Oswalt's equation and divide by b to get

$$a/D = a/b\epsilon + 1/b\epsilon K + 1/\epsilon.$$

They assume that $1/\epsilon$ is small and can be neglected; and by plotting a/D against a for runs at constant b, they can evaluate ϵ and K. It can readily be checked that this is precisely equivalent to assuming that x is negligible compared with a in $(a-x)$ in the original Mass Law equation. This method is

simple to apply, and is sufficiently accurate provided a is appreciably larger than b. It cannot be applied to all mixtures, but it is easy to check whether it is applicable or not. Of course, if b is larger than a, an analogous equation can be derived by ignoring x with respect to b. The Frank and Oswalt equation was valid for all the conditions that were used; but, as it is more difficult to apply, it was only used when its use was unavoidable.

These equations of course assume that no other reactions occur. There are three complicating reactions that must be considered. These are: (i) hydrolysis of Fe^{+3} to $FeOH^{++}$, (ii) formation of thiocyanic acid molecules, and (iii) formation of higher complexes, notably $Fe(SCN)_2^+$. An estimate can be made of the extent to which the first two reactions interfere, and conditions chosen so that this interference is not serious and can be allowed for. The third reaction presumably does not occur at great dilutions; the equilibrium giving the higher complex can be measured by observing the deviations from the simple equations at higher thiocyanate concentrations. The effect of the first two reactions can be estimated as follows.

If K_h is the equilibrium constant for the reaction:

$$Fe^{+3}+2H_2O \rightleftharpoons FeOH^{++}+H_3O^+$$

defined as

$$K_h = (FeOH^{++}) \,.\, (H^+)/(Fe^{+3}),$$

then the total iron concentration is given by

$$a = (Fe^{+3})+(FeOH^{++})+(FeSCN^{++})$$

and $a = (Fe^{+3}) \,.\, \{1+K_h/(H^+)\}+(FeSCN^{++})$.

The Rabinowitch–Stockmayer equation is modified to

$$\frac{a}{D} = \frac{a}{b\epsilon}+\frac{1+K_h/(H^+)}{Kb\epsilon}.$$

Reported values of K_h are given in Table VII. As the extrapolation from low ionic strengths to an ionic strength of 1.2 is somewhat uncertain, the results given above in Table VI were obtained, which show the effect of varying the hydrogen ion concentration in ferric thiocyanate mixtures. These results also provide some evidence on the possible existence of the ion $Fe(OH)(SCN)^+$.

TABLE VII

LITERATURE VALUES OF K_h

All measurements are at 25°C.

K_h	Ionic strength	Method	Reference
0.006	Small	Potentiometric	5
0.0035	Small	Conductivity	12*a*
0.0028	0.05	Spectrophotometric	15*a*
0.0065	Small	Spectrophotometric	20

The interpretation of these results must also take into consideration the possibility of thiocyanic acid formation. If thiocyanic acid is formed the Rabinowitch–Stockmayer equation should be modified much as above:

$$\frac{a}{D} = \frac{a}{b\epsilon} + \frac{1+(H^+)/K_a}{Kb\epsilon}$$

where K_a is the ionization constant of thiocyanic acid. Values of K_a are none too certain, though it is known (15*b*) that thiocyanic acid is a strong acid. Recently Gorman and Connell (9*a*) reported that sodium thiocyanate exerts no discernible buffering action on solutions of perchloric acid. This is confirmed by the results of Table VI in the following way. If the Rabinowitch–Stockmayer equation, allowing for both the above side reactions, is written down:

$$\frac{a}{D} = \frac{a}{b\epsilon} + \frac{(1+K_h/(H^+)) \,.\, (1+(H^+)/K_a)}{Kb\epsilon}$$

and is differentiated to find the point where D is a maximum as (H^+) varies, with a and b constant, we get

$$\frac{\partial D}{\partial (H^+)} = \frac{D^2}{ab\epsilon K}(K_h/(H^+)^2 - 1/K_a).$$

Hence the maximum occurs when $(H^+) = \sqrt{(K_h K_a)}$. The results in Table VI give no definite maximum, but D scarcely increases as (H^+) goes from 0.2 to 0.5, so possibly a maximum occurs at about $(H^+) = 0.5\ M$. The value of K_h (obtained below) at this ionic strength is 1.55×10^{-3}, so K_a is 160 or more. This value of K_a has, of course, no precise quantitative significance, except to show that thiocyanic acid is a strong enough acid for its formation to be ignored. It may be added that the same conclusion about the position of the maximum is reached if the full Mass Law equation is used.

If we turn now to the evaluation of K_h, the Mass Law equation gives:

$$1+K_h/(H^+) = \frac{K}{x}(a-x)(b-x) \cong K\left(\frac{ab}{x} - a - b\right).$$

Calling D_0 the limiting density at high (H^+), this can be rearranged to:

$$K_h = (H^+) \,.\, \left(\frac{D_0}{D} - 1\right) (1+Ka+Kb) \,.$$

Hence to evaluate K_h we must know K. Anticipating later conclusions we can take K as 130. This is not arguing in a circle, since K, as evaluated later, is only slightly dependent on K_h. To obtain (H^+), it is necessary to note that while most of the hydrogen ions come from the added perchloric acid, a little comes from the hydrolysis of the ferric ions. The simplest way to deal with this is by successive approximation, an approximate value of K_h being used to correct (H^+). Table VIII gives the results of this calculation of K_h, based on the experimental results of Table VI. The average value of K_h is 1.55×10^{-3}. To compare this with the literature values which are reported at zero ionic strength some form of extrapolation must be used. Bray and Hershey (5) used an empirical equation, which, if applied to the present data, makes K_h^0 equal to 5.6×10^{-3}. Siddall and Vosburgh (20) used a form of the extended Debye-Hückel equation; with this, K_h^0 comes out at 6.4×10^{-3}. As these are in reasonable agreement with the literature values, our assumptions about the

TABLE VIII

EVALUATION OF K_h

$(H^+)_{uncorr.}$, M	$(H^+)_{corr.}$, M	K_h, $\times 10^{-3}$
0.0175	0.01757	1.61
0.0100	0.01012	1.58
0.0075	0.00765	1.57
0.0050	0.00521	1.77
0.0025	0.00282	1.42
0.00175	0.00214	1.43
0.0010	0.00148	1.44

ions present are presumably correct; in particular it is unlikely that any large concentration of $Fe(SCN)(OH)^+$ is present. This can be checked however by the same type of calculation that will be used later to demonstrate the existence of $Fe(SCN)Cl^+$. When (H^+) falls below 0.005 M, slight, but fairly consistent, deviations occur between the calculated and observed densities. If it is assumed that this is due to the presence of $Fe(SCN)(OH)^+$, the hydrolysis constant of $FeSCN^{++}$ can be calculated. The results do not permit any great accuracy, and the values of the constant vary by $\pm 20\%$, the average being

$$k = \frac{(FeSCNOH^+) \cdot (H^+)}{(FeSCN^{++})} = 6.5(\pm 1) \times 10^{-5}.$$

This means that $FeSCN^{++}$ would be half hydrolyzed at a pH of 4.2, as compared with Fe^{+++} which is half hydrolyzed at a pH of 2.8 at this ionic strength. As is to be expected the larger ion with the smaller charge is hydrolyzed less readily. The extinction coefficient of $Fe(SCN)(OH)^+$ can also be very roughly evaluated, the results being:

λ	4400	4500	4600	4700	4800 Å
ϵ	5400	4500	4600	4500	3900

EVALUATION OF THE DISSOCIATION CONSTANT AND EXTINCTION COEFFICIENTS OF $FeSCN^{++}$

A plot of a/D against a at constant b of the results in Table II gives curves which are very nearly straight lines. This in itself is some evidence of the correctness of this method of treatment. The values of ϵ obtained by the method of least squares from the first two series of runs in Table II are given in Table IX. The two runs agree, with an average deviation of about 1%. This difference

TABLE IX

EVALUATION OF ϵ

ϵ (series 1)	2240	3430	3980	4360	4600	4670	4530	4260	3890	3470	2530
ϵ (series 2)	2270	3450	4010	4460	4670	4730	4590	4370	3940	3480	2530
λ, mμ	400	420	430	440	450	460	470	480	490	500	520

cannot be attributed to the approximations made in deriving the Rabinowitch–Stockmayer equation; it is probably due partly to errors in measuring D, and partly to errors in the concentrations, particularly the very dilute thiocyanate.

Reference to earlier measurements of the same quantity (7, 14) shows appreciable deviation from the present results, though as this earlier work was done

at room temperature part of the difference may be due to different temperatures. Frank and Oswalt's results would agree well with ours, if all their wave lengths were shifted 100 Å to the red. Macdonald reports results that are consistently about 6% higher than ours, and the reason for this inconsistency is not obvious.

Evaluation of K from these data gives the results in Table X. As can be seen there is some range in the values of K, even ignoring the extreme points.

TABLE X

EVALUATION OF K

Wave length used, mμ	K from series 1	K from series 2
400	119	107
420	124	136
430	125	132
440	125	117
450	126	127
460	123	127
470	128	131
480	129	120
490	128	131
500	123	136

This is not due to algebraic approximations, as calculation of a second order correction only decreases K by 0.2%. However some improvement in the method of calculation can be made if ϵ is known, as it can now be taken to be from Table IX. K can be calculated directly from

$$K = \frac{D/\epsilon}{(a-D/\epsilon)(b-D/\epsilon)}.$$

Before calculating K directly, a small correction should be applied to the values of ϵ, because, in any given solution, the value of ϵ at various wave lengths must be proportional to D. The relative values of D at various wave lengths are known from a considerable number of measurements. Accordingly the values taken for ϵ have been smoothed out slightly to agree with the relative values of D; the final result is as follows:

λ	4000	4200	4300	4400	4500	4600	4700	4800	4900	5000	5200 Å
ϵ	2230	3440	3990	4380	4630	4680	4560	4310	3920	3475	2530

This is really applying another test of mutual consistency to our ϵ values, and adjusting them accordingly; the maximum adjustment is 0.7%. Using these ϵ values we can calculate K, with the results given in Table XI. In each run an average K was extracted from all the density readings; some of the readings at the highest thiocyanate concentrations have not been included in the over-all average, because it is evident that higher complexes are becoming important. The final result, corrected for hydrolysis of Fe^{+3}, is $130(\pm 1)$.

TABLE XI

MEAN VALUES OF K FROM EXTINCTION COEFFICIENTS AND OPTICAL DENSITIES

Series	(Fe^{+3}), M	(SCN^-), M	K
3	0.001	0.00025	131.1
		0.00050	131.7
		0.00075	132.2
		0.00100	132.4
		0.00125	134.0
		0.00150	132.6
4	0.002	0.0006	126.2
		0.0008	128.9
		0.0010	131.1
5	0.003	0.0002	128.3
		0.0004	126.6
		0.0006	129.1
		0.0008	129.0
			Mean 130.2±2

Variation of Ionic Strength

The runs reported in Table III were made in order to determine the effect of ionic strength on the dissociation constant and on the extinction coefficients. It seemed likely that the effect of varying ionic strength on the extinction coefficients might be small, but it could not be assumed to be negligible. The extinction coefficients were accordingly measured at the lowest ionic strength used, namely 0.2. In these runs the total thiocyanate ranged from about 1% to 7% of the total iron; as this seemed rather a high amount for the Rabinowitch–Stockmayer equation to hold accurately, the values of ϵ were first calculated by least squares in the usual way, and then a second least squares calculation was made in which the total iron was corrected for the amount present as $FeSCN^{++}$. Finally some of the values were slightly adjusted, as before, to bring them into agreement with the actual absorption spectrum. The results, for an ionic strength of 0.2, were:

λ	4000	4200	4300	4400	4500	4600	4700	4800	4900	5000	5200 Å
ϵ	2280	3490	4030	4415	4625	4670	4530	4250	3875	3420	2480

The results differ from those at an ionic strength of 1.2 by an amount that is little more than the experimental error, but there is a consistent shift of the absorption band to shorter wave lengths at the lower ionic strength: in fact the two bands would virtually coincide if the band at 1.2 ionic strength were shifted 20 Å to the violet.

Since these extinction coefficients were so nearly the same, they were interpolated for runs at intermediate ionic strengths. From these values and the data of Table III, K was calculated to have the values given in Table XII. As before, the values of K drifted at higher thiocyanate concentrations, presumably owing to the formation of $Fe(SCN)_2^+$.

Although it is a long extrapolation from these results to K at zero ionic strength, it is perhaps worth while seeing how well they agree with the extended Debye-Hückel equation:

$$\log K = \log K_0 + \frac{A.\Delta z^2.\sqrt{\mu}}{1+aB\sqrt{\mu}} + C\mu$$

where K_0 is the dissociation constant at zero ionic strength; Δz^2 is the change in the squares of the ionic charges in the reaction, here equal to -6; and A, B, C, and a are constants. If we give A and B their usual values for water of 0.509 and 0.330×10^8, and, following Rabinowitch and Stockmayer (19)

TABLE XII

VALUES OF K AT VARIOUS IONIC STRENGTHS

(SCN^-), M	Ionic strength 0.8	0.5	0.3	0.2
0.00025	133	145	165	189
0.00050	133	146	168	191
0.00075	134	147	169	195
0.00100	134	146	169	192
0.00125	135	149	171	196
Mean	134	146	169	192

and Betts and Dainton (4), if we take a as 4.5×10^{-8} and C as 0.30, we find that our results make K_0 equal to 1070. The degree of fit is best seen by calculating K at various ionic strengths and comparing with experiment:

Ionic strength (μ)	0.2	0.3	0.5	0.8	1.2
K (calculated)	193	165	140	133	140
K (observed)	192	169	146	134	130

While this is not a striking agreement, it is probably as good as could be expected over such a wide range of ionic strengths, and it shows general agreement with the form of the equation.

Temperature Variation

Experiment shows that the optical densities of ferric thiocyanate mixtures are markedly temperature dependent. In general it must be assumed that this is due to changes in both ϵ and K. It is simplest to determine first the dependence of the extinction coefficients on temperature, since it is unlikely that they will change by any large amount. The results given in Table IV for 5°C. and 45°C. enable a determination of ϵ for these temperatures to be made by the Rabinowitch–Stockmayer method; the values so obtained are given in Table XIII. The values at 25°C. are included for comparison. As might be expected the absorption band is slightly narrower at lower temperatures, but this effect is quite small. The chief change is that the maximum extinction coefficient decreases by about 4½% in going from 5°C. to 45°C.; the maximum also shifts slightly to longer wave lengths (about 4570 Å at 5°C. to 4620 Å at 45°C.).

Using these values of ϵ it is possible to calculate K as before. Another series of runs at various intermediate temperatures was also made, the data being also given in Table IV. ϵ was interpolated from the results in Table XIII, and the average values of K for various temperatures were as follows:

Temp.	5	10	15	20	25	30	35	40	45 °C.
K	155	147	139	134	130	126	122	119	118

TABLE XIII

EXTINCTION TEMPERATURES AT VARIOUS TEMPERATURES

λ, mμ	5°C.	25°C.	45°C.
400	2460	2230	2060
420	3550	3440	3230
430	4110	3990	3760
440	4530	4380	4170
450	4710	4630	4440
460	4750	4680	4550
470	4600	4560	4470
480	4320	4310	4260
490	3870	3920	3940
500	3390	3480	3540
520	2400	2530	2670

A plot of log K against $1/T$ gives a somewhat curved line. This is straightened slightly by converting the concentrations used in obtaining K to molal quantities, which are more strictly applicable to calculations of the partial molar heat change in the reaction. In doing this it was assumed that the coefficient of expansion of water was the same as that of the solutions. It is evident that the heat change is small: an average over the range of temperatures gives 0.8 kcal./gm-mol.; and the results suggest that it changes appreciably over the temperature range, which is not inconceivable as it is such a small quantity. However the accuracy of the results did not seem to warrant a more detailed evaluation. If K is converted to K_0 by means of the extended Debye-Hückel equation, making allowance for the variation of the constants in this equation with temperature, we get:

Temp.	5	25	45 °C.
K_0	1090	1070	1100

Hence the heat change at zero ionic strength seems to be virtually zero.

Investigations on Higher Complexes

In almost every investigation dealing with $FeSCN^{++}$ deviations have been found at higher thiocyanate concentrations which have been ascribed to higher complexes (e.g. 3, 7, 18). This explanation is certainly plausible, but direct evidence for it can only be obtained if the dissociation constants of the higher complexes can be evaluated and are found to remain constant. Let us call K_2 the dissociation constant of the second complex, defined by

$$K_2 = \frac{(Fe(SCN)_2^+)}{(FeSCN^{++}) \cdot (SCN^-)}.$$

K_3 will be a similar expression for $Fe(SCN)_3$. Some previous attempts have been made to determine K_2 (with which we are chiefly concerned), but the results have admittedly been very approximate (e.g. 14). Betts and Dainton (4) arrived at a value of 20 by a trial and error method to fit their kinetic data.

We can probably assume that, just as there is a region where only $FeSCN^{++}$ is important, so at somewhat higher concentrations only $FeSCN^{++}$ and $Fe(SCN)_2^+$ are important. The second complex introduces two new constants (K_2 and the second extinction coefficient), and one additional equation (the Mass Law equation for K_2). Because of the complexity of the system, attempts

were made to measure (SCN^-) or (Fe^{+3}) directly by electrochemical means. These were not successful, so the attempts will be only very briefly described.

A silver – silver thiocyanate electrode was prepared essentially by the method of Pearce and Smith (16). Two such electrodes, prepared together, were placed in the two compartments of a cell; one compartment was filled with a sodium thiocyanate solution and the other with an identical solution except for the addition of a known concentration of ferric ions. The two compartments were separated by a sintered glass plug in a narrow tube, and side-arms allowed the cell to be kept filled with nitrogen during a run. The electrodes were moved up and down continuously during a run. The cell was immersed in a water thermostat, and its e.m.f. was measured on a Leeds and Northrup K2 potentiometer. In trial runs, using different thiocyanate concentrations in the two compartments, the cell quickly settled down to give steady readings which varied by less than 0.1 millivolt in an hour. Unfortunately when ferric perchlorate was introduced, the results were never sufficiently steady to be useful. It was calculated that only an e.m.f. steady to less than 0.1 mv. would be useful; and it was found after considerable trial that the e.m.f. did not stay constant and reproducible to this accuracy. A ferrous–ferric cell and polarographic methods were also tried, but did not prove accurate enough.

Another type of attack was made on the same problem by adding some other ion in small amount which forms a compound with either ferric or thiocyanate ions, and measuring the effect of this on the optical density. If the dissociation constant of the complex so formed is known, we have enough information to calculate all the concentrations in the solution. Chloride was tried, since the dissociation constant of $FeCl^{++}$ is known; unfortunately there are definite indications that $Fe(SCN)Cl^+$ is also formed, and this vitiates the calculation. The behavior of mixtures of this sort will be reported in a later paper. Ions which form complexes with thiocyanate were also tried. Monovalent thallium gives TlSCN, whose dissociation constant has been measured by Bell and George (2), but this gave no absorption spectrum within the range of the spectrophotometer. Cobalt (11), chromium (111), and molybdenum (111) produce complex ions, but it was found that their absorption spectrum overlaps too much with that of Fe^{+3} or $FeSCN^{++}$. Nickel (11) and manganese (11) were tried, but gave no absorption that could be attributed to a thiocyanate complex.

Finally spectrophotometric measurements were made, as for $FeSCN^{++}$, and the results interpreted in a way that, it is believed, gives a good, though slightly approximate, value for K_2 and ϵ_2, the second extinction coefficient. The data are given in Table V. The upper limit to the thiocyanate concentration is set by the fading of the sort investigated by Betts and Dainton (4); this is a different reaction from that encountered earlier, which was retarded by benzyl alcohol. At high concentrations fading is due to the reduction of ferric to ferrous ions by thiocyanate. Hydrogen peroxide prevents this (or reoxidizes the ferrous ions) up to a thiocyanate concentration of about 0.2 *M*.

The results show the previously observed shift of the maximum to longer

wave lengths; and the densities are greater than if only $FeSCN^{++}$ had been formed. To interpret the results, we note that there are eight quantities that we need to know: these are the four concentrations of Fe^{+3}, SCN^-, $FeSCN^{++}$, and $Fe(SCN)_2^+$, and the four constants, namely K, K_2, ϵ, and ϵ_2. K and ϵ have, of course, already been measured. We have five equations available: the Mass Law expressions for K and K_2, the two mass balances for total iron and thiocyanate, and Beer's law. We can eliminate the four concentrations from these five equations, and obtain a single equation in K, K_2, ϵ, and ϵ_2. By varying the concentrations, and hence D, we can get this equation with various known numerical coefficients; from any two such equations we can evaluate K_2 and ϵ_2. Thus it is possible in theory to disentangle the constants directly, but the algebra is so cumbersome that a method of successive approximations was used. It was assumed as a first approximation that

$$\epsilon_2 = 2.\epsilon,$$

i.e. that the absorption of an ion is proportional to the number of thiocyanate radicals attached. If this is so, then

$$[i] \qquad (SCN^-) = b - D/\epsilon$$

where b, as before, is the total thiocyanate present. The mass balance for iron can be put in the form:

$$a = (Fe^{+3}).\{1+K(SCN^-)+K.K_2(SCN^-)^2\},$$

from which we get:

$$D\{1+K(SCN^-)+K.K_2(SCN^-)^2\}/a = \epsilon K(SCN^-)+\epsilon_2 K.K_2(SCN^-)^2.$$

K_2 was adjusted in the left-hand side of this equation, with the use also of the value of (SCN^-) from equation [i] above, until the values of $\epsilon_2 K.K_2$ showed minimum deviation. The best value of K_2 was $15(\pm 1)$. The values of K_2 were effectively constant as (SCN^-) varied from 10^{-2} to 10^{-1} molar. As an example of the constancy obtained, the average deviation of the values obtained at a wave length of 4600 Å over this range was 0.2×10^6 for $\epsilon_2 K.K_2$, while the average value of $\epsilon_2 K.K_2$ was 17.3×10^6. This is an average deviation of about 1%.

This average value makes ϵ_2 equal to 8900 at 4600 Å, so $\epsilon_2 = 1.90\epsilon$. The first approximation that $\epsilon_2 = 2.\epsilon$ is altered only 5% for the next approximation. If we substitute this new value of ϵ_2, K_2 is raised by 3%. This method is equivalent to calculating (SCN^-) from K and the approximate value of K_2. As little thiocyanate is used up in forming the second complex, (SCN^-) is very little altered by any error in K_2. If we know (SCN^-), we can substitute it in the equations and find ϵ_2 and K_2 directly. Doing this gave a value of K_2 only $\frac{1}{3}$% different from the second approximation, and a value of ϵ_2 of 9130 at 4600Å. This is probably the best approximate method. The final value for K_2 was $15\frac{1}{2}(\pm 1)$.

Values of ϵ_2 at other wave lengths were obtained in a similar manner. The results were:

λ	4200	4400	4500	4600	4700	4800	4900	5000 Å
ϵ_2	6550	8030	8700	9130	9740	9980	10080	9740

It is interesting to note that the absorption maximum is shifted towards the red to 4870 Å.

At very high thiocyanate concentrations the calculated values of K_2 start to drift upwards; this is due, doubtless, to appreciable amounts of $Fe(SCN)_3$ being formed. If we include this possibility in our equations, and assume that the extinction coefficient of $Fe(SCN)_3$ is three times that of $FeSCN^{++}$ (this is analogous to our first approximation for $Fe(SCN)_2^+$), the value of K_3 comes out close to 1. K_3 is here defined as

$$K_3 = \frac{(Fe(SCN)_3)}{(Fe(SCN)_2^+) \cdot (SCN^-)}.$$

This is necessarily very rough, but it was impossible to continue measurements to higher thiocyanate concentrations.

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FERRIC SULPHATE COMPLEXES, AND TERNARY COMPLEXES WITH THIOCYANATE IONS[1]

By M. W. Lister and D. E. Rivington

ABSTRACT

A spectrophotometric study of solutions containing ferric and sulphate ions, and also ferric, sulphate, and thiocyanate ions, is reported. The measurements were made at constant temperature and ionic strength; the effect of varying acidity was also investigated. The data were interpreted with the help of the results of the first paper of this series. It is believed that the data show that $FeSO_4^+$ and $FeHSO_4^{++}$ ions are formed. Values are obtained for the equilibrium constants for their formation from simple ions, and for their extinction coefficients. At higher sulphate concentrations there is evidence for the appearance of $Fe(SO_4)_2^-$ and $FeSO_4.HSO_4$, and estimates are made of their equilibrium constants. When thiocyanate is also present, the data can best be interpreted by assuming that the complex $FeSO_4.SCN$ is formed, and at higher sulphate probably also $Fe(SO_4)_2SCN^{--}$. Values are obtained for the equilibrium constants for the formation of these species, and the results would indicate that mixed complexes of this type are formed as readily as 'simple' ones such as $Fe(SO_4)_2^-$.

INTRODUCTION

One of the possible methods for investigating the complexes of ferric ions is to use thiocyanate as an indicator ion. It is generally observed that the red color of a ferric thiocyanate solution fades as another ion is added which also forms a complex with ferric ions; and by suitable colorimetric measurements it should be possible to determine the equilibrium constant of the formation of this other complex. This method has been employed in a few investigations, e.g. by Lanford and Kiehl (6) using phosphate ions; and it was also used in effect by Bent and French (1) for chloride ions in their early paper on ferric thiocyanate. In the latter case the equilibrium constant of the reaction $Fe^{+++}+Cl^- \rightleftharpoons FeCl^{++}$, as obtained with the help of thiocyanate, differs from the result obtained directly without thiocyanate. This matter has been investigated by the present authors, and will be reported in another paper. The present paper reports work on the application of this method to ferric sulphate complexes. Investigations of the sulphate complexes have been described in two papers appearing since the present work was started, though in neither case was the thiocyanate ion used as indicator. The first paper by Sykes (9) reported measurements on the effect of sulphate ions on the kinetics of the reaction between ferric and iodide ions: Sykes concluded that the equilibrium constant for $FeSO_4^+$,

$$k_1 = (FeSO_4^+)/(Fe^{+++}) \cdot (SO_4^{--}),$$

had a value of 15,000 at zero ionic strength. He did not investigate the ion $FeHSO_4^{++}$. Whiteker and Davidson (10) in the second paper decided that $FeHSO_4^{++}$ did not occur in significant amounts because of the rather small differences produced in the absorption spectra of ferric sulphate systems by alteration of the hydrogen ion concentration. On this basis they evaluated

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Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario.

k_1 and found it to be 105 ± 15. The evidence against $FeHSO_4^{++}$ did not appear to be quite conclusive, and it was decided to investigate this particular matter in more detail. This was done directly on solutions of ferric and sulphate ions at different acidities. The alternative method of using thiocyanate as an indicator ion was also carefully examined, and it is believed that the results show that ternary complexes containing ferric, sulphate, and thiocyanate ions must be present. It is evident that similar work with other ions must also take into account the possible formation of ternary complexes.

APPARATUS AND MATERIALS

The extent of formation of the various complex ions was determined from the optical densities of the solutions as measured on a Beckmann DU spectrophotometer. The techniques used were generally the same as those used in the first paper of this series (7). The modification of the spectrophotometer and the methods of preparing the solutions were identical. The chemicals used were also the same, with the single addition of sulphuric acid, of which a stock solution was made by diluting the reagent grade acid.

EXPERIMENTAL RESULTS

As in the first paper of the series (7), runs were carried out at 25°C., and an ionic strength of 1.2, which was controlled by the addition of sodium perchlorate. Three series of runs were made. (i) Runs were made in which the ferric ion concentration was varied but the sulphate kept constant. The ferric ion was in considerable excess, so the results could be interpreted by the method devised by Rabinowitch and Stockmayer (8), though some extension of the method was necessary owing to the greater complexity of the sulphate systems.

TABLE I

OPTICAL DENSITIES OF FERRIC SULPHATE SOLUTIONS

Light path = 1 cm.; sulphate concentration = 0.001 *M* in all cases; ionic strength = 1.2

(Fe^{+3}), *M*	(H^+), *M*	Wave length, mμ					
		360	350	345	340	335	330
0.005	0.50	.019	.030	.037	.046	.055	.065
0.010		.035	.057	.071	.090	.106	.125
0.015		.045	.076	.099	.120	.145	.171
0.020		.060	.100	.124	.154	.183	.211
0.025		.069	.115	.144	.175	.209	.237
0.030		.080	.131	.165	.199	.226	.246
0.005	0.20	.034	.056	.071	.089	.106	.125
0.010		.060	.101	.126	.156	.190	.221
0.015		.084	.140	.175	.215	.255	.299
0.020		.100	.166	.207	.254	.301	.342
0.025		.115	.190	.236	.285	.330	.360
0.005	0.10	.049	.080	.102	.125	.151	.179
0.010		.086	.141	.179	.219	.261	.305
0.015		.112	.186	.233	.284	.335	.385
0.020		.127	.213	.262	.319	.369	.409
0.005	0.05	.060	.102	.128	.157	.190	.224
0.010		.099	.164	.205	.253	.300	.349
0.015		.120	.200	.247	.299	.346	.379

TABLE II

DEPENDENCE OF OPTICAL DENSITY ON (H^+) IN FERRIC SULPHATE SOLUTIONS
$(Fe^{+3}) = 0.001\ M$; $(SO_4^{--}) = 0.050\ M$; 25°C.; ionic strength = 1.2

	Wave length, mμ				
(H^+), M	350	345	340	335	330
0.20	.394	.491	.603	.722	.840
0.10	.458	.577	.709	.842	.974
0.05	.500	.631	.773	.920	1.058
0.02	.510	.642	.790	.939	1.073

TABLE III

OPTICAL DENSITIES OF SOLUTIONS CONTAINING FERRIC, THIOCYANATE, AND SULPHATE IONS
$(Fe^{+3}) = 0.001\ M$; $(SCN^-) = 0.001\ M$; $(H^+) = 0.2\ M$; 25°C.; ionic strength 1.2

	Wave length, mμ				
(SO_4^{--}), M	440	450	460	470	480
0	.450	.471	.480	.468	.443
0.0025	.430	.452	.460	.449	.423
0.005	.420	.441	.448	.437	.411
0.0075	.401	.421	.428	.417	.390
0.010	.387	.409	.412	.401	.377
0.0125	.372	.391	.396	.385	.363
0.015	.364	.381	.386	.376	.354
0.0175	.356	.375	.378	.367	.346
0.020	.342	.359	.361	.351	.330
0.025	.325	.339	.342	.331	.311
0.030	.309	.323	.325	.316	.295
0.035	.295	.307	.309	.299	.280
0.040	.280	.292	.294	.281	.265
0.050	.255	.265	.266	.256	.239

Various runs were made at different hydrogen ion concentrations, so that the possible formation of $FeHSO_4^{++}$ could be investigated. The results are given in Table I. (ii) A short series of runs were made at higher sulphate and lower ferric ion concentrations, with varying hydrogen ion concentrations. The results throw some light on the formation of higher complexes, and are given in Table II. (iii) A series were made with constant (and low) ferric and thiocyanate ion concentrations, and with sulphate also present in a fairly wide range of concentrations. The acidity was kept constant. This series gave a comparison of the equilibrium constants obtained with and without thiocyanate. The results are given in Table III.

DISCUSSION OF RESULTS

A discussion of the results in Table I should perhaps be prefaced by saying that the optical densities in the table are relative to solutions of identical composition except that no sulphate has been added. Qualitatively an examination of the solutions containing sulphate shows an absorption band with a maximum at 3050 Å, which is absent without sulphate. However ferric perchlorate solutions are themselves beginning to absorb at this wave length, so the measurements were made at wave lengths from 3300 to 3600 Å, where ferric perchlorate scarcely absorbs at all. Hence there is no doubt that the absorption is really due to ferric sulphate complexes.

It is apparent from Table I that a high hydrogen ion concentration reduces the optical density of ferric sulphate solutions, but that the relative densities at different wave lengths are scarcely altered. Hence the effect of hydrogen ion is either just to remove absorbing ions (presumably $FeSO_4^+$), or to introduce other ions of similar spectrum (presumably $FeHSO_4^{++}$), or both. To explain the results it is necessary to consider various possible equilibria. It seemed reasonable to suppose that, under the conditions of the first run (0.001 *M* sulphate, 0.005 to 0.03 *M* iron, and varying acidity), the following equilibria might be important:

$$Fe^{+++} + SO_4^{--} \rightleftharpoons FeSO_4^+,$$
$$Fe^{+++} + HSO_4^- \rightleftharpoons FeHSO_4^{++},$$
$$H^+ + SO_4^{--} \rightleftharpoons HSO_4^-.$$

The acidity was high enough to suppress the formation of $FeOH^{++}$ to more than a small extent. It was thought unlikely that ions containing two iron atoms would be formed (e.g. $Fe_2SO_4^{++}$), chiefly because in general it never seems necessary to postulate such ions in fairly dilute aqueous solutions; so this possibility was ignored.

The results in Table I can be treated by an extension of the Rabinowitch and Stockmayer (8) method. This method assumes that only a negligible fraction of the iron is complexed, the concentrations being such that this is a reasonably good approximation. If we represent the concentrations of the various ions by the following symbols:

$$p = (Fe^{+++}), \quad q = (SO_4^{--}), \quad r = (HSO_4^-),$$
$$x = (FeSO_4^+), \quad y = (FeHSO_4^{++}), \quad s = (H^+),$$

and a = total iron present,
b = total sulphate present,
c = total acid put in, measured in hydrogen ion concentration;

then: $x/pq = k_1$ (equilibrium constant for $FeSO_4^+$),
$y/pr = k_m$ (equilibrium constant for $FeHSO_4^{++}$),
$sq/r = k_a$ (ionization constant for HSO_4^-);

and for the mass balances:

$$a = p+x+y,$$
$$b = q+r+x+y,$$
$$c = s+r+y.$$

For the optical density, D, we have:

$$D = \epsilon_1 x + \epsilon_m y,$$

where ϵ_1 is the extinction coefficient of $FeSO_4^+$,
and ϵ_m is the extinction coefficient of $FeHSO_4^{++}$.

As a first approximation we can write $a = p$, i.e. little iron is complexed, and $c = s$, as only a little sulphate is present, so the formation of bisulphate ions

will not significantly alter the hydrogen ion concentration. The first assumption is equivalent to that made by Rabinowitch and Stockmayer. We can now rapidly eliminate p, r, s, x, and y from the above equations, when we get:

$$b = q(1+c/k_a+k_1a+k_mca/k_a),$$
$$D = aq(\epsilon_1 k_1+\epsilon_m k_m c/k_a).$$

Finally, eliminating q, and rearranging, we get:

[i] $$\frac{D}{a}\left(1+\frac{c}{k_a}\right) = k_1(b\epsilon_1-D)+k_m(b\epsilon_m-D)\frac{c}{k_a}.$$

This means that at any given wave length a plot of D/a against D should give a straight line. The slope of this line is given by:

[ii] $$\frac{\partial(D/a)}{\partial D} = -\frac{(k_1k_a+k_mc)}{k_a+c}.$$

Applying this to the data of Table I, moderately straight lines result; the straightness is best at fairly high acidity and not too high total iron. Averaging the data for all wave lengths, we get the results given below.

c	0.5	0.2	0.1	0.05
Average slope	16.5	28.5	46.3	71.0
Range of slope	14.9–18.5	26.9–30.9	42.8–49.1	63.5–79.2

By 'slope' is meant $-\partial(D/a)/\partial D$. There was a tendency for the slope to increase at higher values of a; where this was noticeable the slope was taken from the best line for the earlier points. 'Range of slope' gives the range over different wave lengths: there was no observable trend with wave length.

To evaluate k_1 and k_m, we must first know k_a. Various values of this constant have been reported (see for example Harned and Owen (5)), but the most reliable value is probably that from a critical survey of the data made by Davies, Jones, and Monk (3). They conclude that at zero ionic strength $k_a = 0.010$. This value is close to the more careful early estimates (4). The value of this constant at the ionic strength used here is open to some doubt, but fortunately the results in the present paper provide some check on the figure chosen. If we use the extended form of the Debye-Hückel equation, for instance in the form proposed by Davies (2), we get $k_a = 0.038$ at an ionic strength of 1.2. This extrapolation is too long for us to be able to conclude more than that a value near 0.04 would be plausible. Young and Blatz (11) from Raman spectra obtain values for the concentrations of sulphate and bisulphate ions in solutions of sulphuric acid. Taking solutions of the same ionic strength, their results would make k_a lie between 0.04 and 0.05 at an ionic strength of 1.2. It therefore seems probable that k_a should be taken as near 0.04.

Referring back to equation [ii], if we call the slope S, then

$$S(1+k_a/c) = k_1 k_a/c+k_m.$$

Hence a plot of $S(1+k_a/c)$ against k_a/c should give a straight line. If this plot is made, the result is reasonably straight for $k_a = 0.03$ and 0.04, but is definitely

curved for $k_a = 0.02$ or 0.05. The present results are not accurate enough to give more than a very rough value of k_a, but they are consistent with a value in the range 0.03 to 0.04. The values of k_1 and k_m that are obtained are:

k_a	0.03	0.04
k_1	178	153
k_m	6.8	5.6

We can therefore conclude that at this ionic strength, $k_1 = 165(\pm 10)$ and $k_m = 6(\pm 1)$. It seems definite that k_m is not zero, and that the species $FeHSO_4^{++}$ is present. It is also possible to calculate the ionization constant of $FeHSO_4^{++}$ considered as an acid: $FeHSO_4^{++} \rightleftharpoons FeSO_4^{+} + H^{+}$. The ionization constant equals $k_m/k_1 k_a$, which comes out close to unity. This is at least a reasonable figure, since $FeHSO_4^{++}$ would probably be a stronger acid than HSO_4^{-}.

If we take these values of k_1 and k_m, we can substitute in equation [i], and get a number of equations in ϵ_1 and ϵ_m at any wave length. From any pair of these, ϵ_1 and ϵ_m can be obtained separately with the following results:

λ	3600	3500	3450	3400	3350	3300 Å
ϵ_1	270	440	560	690	810	970
ϵ_m	160	265	340	390	470	560

It is interesting to note that ϵ_m is always about three fifths of ϵ_1. These extinctions might be expected to run parallel, but there seems to be no obvious reason for this ratio.

If we look now at the results in Table II, at relatively high sulphate, a rough calculation shows that the densities do not agree adequately with the densities calculated from the assumptions made in treating Table I. This presumably means that other species are present; the most probable one is $Fe(SO_4)_2^{-}$, and possibly $Fe(SO_4)(HSO_4)$. The rigorous treatment of a mixture of this sort is not easy, but we can make simplifying assumptions. As the mixtures in Table II all contained 0.001 M total iron, and 0.05 M total sulphate, a plausible approximation is that:

$$b = q + r,$$

i.e. the sulphate is almost all present as SO_4^{--} or HSO_4^{-}. The total acid added is also much more than the total iron, so approximately:

$$c = s + r,$$

i.e. the acid hydrogen is present either as H^{+} or as HSO_4^{-}. These approximations, and the ionization constant of HSO_4^{-} ($k_a = sq/r$), enable us to calculate s, q, and r. If we suppose that $Fe(SO_4)_2^{-}$ is the only new ion formed, and call its concentration z, its extinction coefficient ϵ_2, and its equilibrium constant k_2, where

$$k_2 = \frac{[Fe(SO_4)_2^{-}]}{[Fe^{+3}][SO_4^{--}]^2} = z/pq^2,$$

then we have from the mass balance for iron:

$$a = p + x + y + z$$

and for the optical density:

$$D = \epsilon_1 x + \epsilon_m y + \epsilon_2 z.$$

Eliminating p, x, y, and z, the result is:

$$a(\epsilon_1 k_1 q + \epsilon_m k_m r + \epsilon_2 k_2 q^2) = D(1 + k_1 q + k_m r + k_2 q^2).$$

Hence from the data in Table II, we get a series of equations in the two unknowns ϵ_2 and k_2. This equation is, in fact, of the form:

$$\epsilon_2 = \alpha/k_2 + D/a$$

where α is a function of known quantities. Values of α and D/a were calculated, and then by combining them in pairs with the result at $c = 0.1$, the following values for k_2 were obtained:

	Wave length, Å				
c	3500	3450	3400	3350	3300
0.2	3.24	2.78	2.79	3.14	3.13×10^4
0.05	1.67	1.58	1.67	1.65	1.68×10^4
0.02	1.72	1.69	1.67	1.69	1.62×10^4

The results at $c = 0.05$ and 0.02 are fairly constant and make $k_2 = 16{,}600$ as an average. The values at $c = 0.2$ give a discrepancy, although this discrepancy is not as big as it looks, since a change of only about 0.02 in the density readings would bring these points also into line. However we are faced with the choice that (i) our general picture of these mixtures is wrong, and the results in Tables I and II must be reconciled by postulating some other ionic species; or (ii) some other new species is beginning to make its appearance at $c = 0.2$ (high acidity). One is naturally loath to reject the idea that $Fe(SO_4)_2^-$ ions are responsible for the deviations in the optical densities at high sulphate concentrations, since this is the obvious explanation. Hence it seems most probable that the explanation is that at $c = 0.2$ the new species $Fe(SO_4)(HSO_4)$ is beginning to appear. A rough calculation can be made of the amount of this species present on the assumption that its extinction coefficient is equal to that of $Fe(SO_4)_2^-$ (see below), which is probably at least roughly true. The optical density at $c = 0.2$ was calculated assuming no $Fe(SO_4)(HSO_4)$. The results at different wave lengths were moderately consistent: the calculated concentrations ranged from 2.7 to 3.7×10^{-5} with a mean of 3.3×10^{-5}. This makes the equilibrium constant for the reaction

$$Fe(SO_4)(HSO_4) \rightleftharpoons Fe^{+3} + SO_4^{--} + HSO_4^-,$$

defined as

$$k_x = \frac{[FeSO_4.HSO_4]}{[Fe^{+3}][SO_4^{--}][HSO_4^-]},$$

about 380 (perhaps ± 50). Considered as an acid the ionization constant of $FeSO_4.HSO_4$ would be about 1.7, which is not far from that for $FeHSO_4^{++}$.

The extinction coefficients of $Fe(SO_4)_2^-$ are readily calculated once k_2 is known; the results are:

λ	3500	3450	3400	3350	3300 Å
ϵ_2	560	700	860	1030	1170

These extinction coefficients are parallel to ϵ_1 and are about 1.25 times as large.

Summarizing this part, it seems fair to say that the picture presented is simple and fairly consistent, and it is unlikely that an equally satisfactory alternative explanation could be found. The results show that $FeSO_4^+$ is first formed (equilibrium constant 165, or 170 to two significant figures), and it seems impossible to interpret the effect of acid unless we also suppose that $FeHSO_4^{++}$ is also formed. At higher concentrations of sulphate $Fe(SO_4)_2^-$ is formed, and perhaps $FeSO_4.HSO_4$. The extinction coefficients of the three species $FeSO_4^+$, $FeHSO_4^{++}$, and $Fe(SO_4)_2^-$ run nearly parallel.

Table III reports the densities of solutions containing ferric, sulphate, and thiocyanate ions. The first point to decide is whether such solutions could be used to obtain the equilibrium constant for $FeSO_4^+$. A rough examination of the results showed that in fact they did not give the same value for k_1, at least assuming that the only complex ions present are $FeSO_4^+$, $FeSCN^{++}$, and $FeHSO_4^{++}$. We are therefore driven to supposing that mixed complex ions are formed, such as $FeSO_4.SCN$. This situation is found to be repeated in systems containing ferric, thiocyanate, and chloride or bromide ions. Examination of the results of Table III also show the following: (i) the relative densities at different wave lengths for any given sulphate concentration stay very much the same throughout; (ii) at any one wave length the density falls off as sulphate is added, and at first the fall in density is proportional to the sulphate added; and (iii) at higher sulphate concentrations the fall in density is less rapid than at first.

We can discover the extent to which $FeSO_4.SCN$ is formed from the following considerations. Let us use the same symbols for the concentrations of various species with the following additions:

$$(SCN^-) = n, \quad (FeSCN^{++}) = m, \quad (FeSO_4SCN) = j,$$

and let us write for the equilibrium constants:

$$\frac{(FeSCN^{++})}{(Fe^{+3})(SCN^-)} = k_t, \quad \frac{(FeSO_4.SCN)}{(Fe^{+3})(SO_4^{--})(SCN^-)} = k_j.$$

As these measurements were made at wave lengths from 4400 to 4800 Å where the simple sulphate complexes scarcely absorb, the optical density is given by

$$D = \epsilon_j j + \epsilon_t m$$

where ϵ_j and ϵ_t are the extinction coefficients of $FeSCN^{++}$ and $FeSO_4.SCN$. As before, let a, b, and c represent the total concentrations of iron, sulphate, and acid hydrogen, and in addition let d be the total concentration of thiocyanate. As c is much larger than a, we shall assume as before that $c = s + r$. Also, when b is small, it seems reasonable to assume that no ions containing

two sulphates have yet been formed. Thus the following nine species may be present: Fe^{+3}, SO_4^{--}, HSO_4^-, $FeSO_4^+$, $FeHSO_4^{++}$, SCN^-, $FeSCN^{++}$, $FeSO_4.SCN$, and H^+. There are five Mass Law equations:

$$k_a = sq/r, \quad k_1 = x/pq, \quad k_m = y/pr, \quad k_i = m/pn, \quad k_j = j/pnq;$$

and four equations for the mass balances:

$$\begin{aligned} a &= p+x+y+m+j, \\ b &= q+r+x+y+j, \\ c &= s+r, \\ d &= n+m+j; \end{aligned}$$

and the equation, given above, for the optical density. Hence, in theory, the nine concentrations can be eliminated, and values of all the constants obtained by combining a sufficient number of measurements. It is not difficult to eliminate x, y, m, j, s, and r to get

$$\begin{aligned} a &= p\left[1+k_1q+\frac{k_mcq}{k_a+q}+n(k_i+k_jq)\right], \\ b &= q\left[1+\frac{c}{k_a+q}+k_1p+\frac{k_mcp}{k_a+q}+k_jpn\right], \\ n &= p\left(1+k_1q+\frac{k_mcq}{k_a+q}\right), \\ D &= pn(\epsilon_i k_i+\epsilon_j k_jq). \end{aligned}$$

These equations also use the fact that in Table III, $a = d$. When b is small, so is q, and we can at first ignore q compared with k_a. If we also call $k_1+k_m c/k_a=\alpha$, these equations reduce to:

$$\begin{aligned} a &= p[1+\alpha q+n(k_i+k_j q)], \\ b &= q(1+c/k_a+\alpha p+k_j pn), \\ n &= p(1+\alpha q), \\ D &= pn(\epsilon_i k_i+\epsilon_j k_j q). \end{aligned}$$

Let p_0, n_0, and D_0 be the values of these quantities when $b = 0$. Then since $q_0 = 0$

$$\begin{aligned} a &= p_0(1+k_i n_0), \\ n_0 &= p_0, \\ D_0 &= p_0 n_0 \epsilon_i k_i. \end{aligned}$$

If we differentiate the above equations with respect to b, and suppose that $b = 0$, we get equations giving the initial slopes of the quantities p, q, n, and D as sulphate is added. The resulting equations (with a little rearrangement) are:

$$\left(\frac{\partial q}{\partial b}\right)_0 = \frac{1}{1+c/k_a+\alpha p_0+k_j p_0^2},$$

$$\left(\frac{\partial p}{\partial b}\right)_0 = -\frac{(a\alpha+k_j p_0^2)}{(1+2k_i p_0)}\left(\frac{\partial q}{\partial b}\right)_0,$$

$$\frac{1}{D_0}\left(\frac{\partial D}{\partial b}\right)_0 = \frac{2}{p_0}\left(\frac{\partial p}{\partial b}\right)_0+\left(\alpha+\frac{\epsilon_j k_j}{\epsilon_i k_i}\right)\left(\frac{\partial q}{\partial b}\right)_0.$$

As $(\partial D/\partial b)_0$ is known from the data, and as $(\partial q/\partial b)_0$ and $(\partial p/\partial b)_0$ could be eliminated, we have in effect at each wave length an equation containing the unknowns ϵ_j and k_j. Since the shape of the absorption curve is virtually unaltered by adding sulphate, ϵ_j is presumably proportional to ϵ_i; and from the nature of the species concerned it seems reasonable to take $\epsilon_j = \epsilon_i$ as a first approximation. This last approximation can be checked to some extent from the results as more sulphate is added; but the effect of other ions, notably $Fe(SO_4)_2^-$, makes the whole system so complicated that this confirmation is not at all strong.

The observed values of $(1/D_0)(\partial D/\partial b)_0$, taken over the range $b = 0$ to 0.01, are:

λ	4400	4500	4600	4700	4800 Å
$(1/D_0)(\partial D/\partial b)_0$	−14.0	−14.1	−14.2	−14.3	−14.9

Probably the best average is −14.2. Substituting this in the equations above the value of k_j comes out at 11,500. The magnitude of this constant is not unexpected; since, combining it with other known constants, it means that the reaction

$$FeSO_4^+ + SCN^- \rightleftharpoons FeSO_4.SCN$$

has an equilibrium constant of 70; and the reaction

$$FeSCN^{++} + SO_4^{--} \rightleftharpoons FeSO_4.SCN$$

has an equilibrium constant of about 90. No very great accuracy can be claimed for k_j, but it probably lies between 10,000 and 13,000.

As the sulphate is increased, the optical density falls off linearly at first and then less rapidly. If we calculate the concentrations of the various species, assuming that no new ions are formed and that $\epsilon_i = \epsilon_j$, the results make a plot of D against b deviate only quite slowly from a straight line (the fact that a straight line is maintained for a moderate distance is some evidence that our assumptions about the solutions containing sulphate up to about 0.01 M were right). The deviation from this straight line must mean that some new colored ion is appearing that is competing successfully against the ions $Fe(SO_4)_2^-$ and $FeSO_4.HSO_4$ which will now be present. This was checked in another way. At higher sulphate concentrations (>0.02) the fraction of sulphate used up in complexes will be so small that we can write approximately:

$$b = q + r.$$

As $c = s + r$, and $k_a = sq/r$, this enables us to find q, r, and s. Allowing now for the presence of $Fe(SO_4)_2^-$ and $FeSO_4HSO_4$, it is possible to calculate D in terms of q and known constants. The observed densities were greater than the calculated ones. As mentioned above, the calculated curve only departs very slowly from a straight line. The only plausible new colored (at 4600 Å) ion is $Fe(SO_4)_2.SCN^{--}$, and a rough estimate of the extent to which it might be present was made in the following way.

If we assume that the species $Fe(SO_4)_2^-$ and $FeSO_4.HSO_4$ may now also be present, but not $Fe(SO_4)_2.SCN^{--}$, the equations for the various concentrations can be reduced to the following:

$$a = p[f(q)+n(k_i+k_j q)],$$
$$n = p.f(q),$$
$$D = pn(\epsilon_i k_i+\epsilon_j k_j q),$$

where

$$f(q) = 1+\left(k_1+\frac{k_m c}{k_a+q}\right) q+k_2\left(1+\frac{k_a c}{k_i(k_a+q)}\right) q^2.$$

k_i is the ionization constant of $FeSO_4.HSO_4$ as an acid. If we assume as above that $\epsilon_i = \epsilon_j$, and call $D/\epsilon_i = X$, we get

$$(a-X)^2 = Xf(q)/(k_i+k_j q).$$

As q is known, X can be calculated. The results of this calculation can be compared with experiment as in Table IV. There is evidently a very slight

TABLE IV

VALUES OF $10^5\ X$

	Wave length							
b	440	450	460	470	480	Mean X_{obs}	X_{calc}	Difference
0.025	7.42	7.32	7.31	7.26	7.22	7.31	7.03	0.28
0.03	7.05	6.98	6.94	6.93	6.84	6.95	6.49	0.46
0.035	6.74	6.63	6.60	6.56	6.50	6.61	6.00	0.61
0.04	6.39	6.31	6.28	6.16	6.15	6.26	5.56	0.70
0.05	5.82	5.72	5.68	5.61	5.55	5.68	4.80	0.88

shift of the absorption spectrum to the violet, the maximum being shifted about 30 Å. However as points on both sides of the maximum have been used, it is probably legitimate to compare a mean value of X as observed with the calculated X. The difference is given in the last column, and this may tentatively be taken as the concentration of $Fe(SO_4)_2.SCN^{--}$. This involves the assumptions (i) that the extinction coefficient of $Fe(SO_4)_2.SCN^{--}$ is the same as that of $FeSCN^{++}$ at these wave lengths, and (ii) that the amount of $Fe(SO_4)_2.SCN^{--}$ present is not enough to throw out the calculations of the concentrations of the other ions present by any great amount. The first assumption is fairly reasonable from the formulae of the ions, but its only other justification is that it gives a plausible and moderately constant value of the equilibrium constant of this ion. The second assumption is at least nearly true, since even at $b = 0.05$ the concentration of ferric ions is about 24 times, and that of thiocyanate ions is 110 times as great as that of $Fe(SO_4)_2.SCN^{--}$. The relevant concentrations are given in Table V. The last column gives the

TABLE V

b	$(Fe(SO_4)_2SCN^{--})$, M	(Fe^{+3}), M	(SO_4^{--}), M	(SCN^-), M	k_w
0.025	0.28×10^{-5}	4.19×10^{-4}	4.37×10^{-3}	9.30×10^{-4}	3.76×10^5
0.03	0.46	3.63	5.34	9.35	4.75
0.035	0.61	3.15	6.35	9.40	5.12
0.04	0.70	2.74	7.40	9.44	4.95
0.05	0.88	2.09	9.61	9.52	4.79

equilibrium constant:

$$k_w = \frac{(Fe(SO_4)_2.SCN^{--})}{(Fe^{+3})(SO_4^{--})^2(SCN^-)}.$$

The values obtained for it are fairly consistent, and the last four values average 490,000. This means that the equilibrium constant for the attachment of a second sulphate to $FeSO_4.SCN$ is 43, and for the attachment of a thiocyanate ion to $Fe(SO_4)_2^-$ is 29. These values are reasonably similar to the corresponding values for attachment of sulphate and thiocyanate ions to other complex ferric ions; however the constants obtained will be reviewed after results with chloride and bromide have been reported. In conclusion it may be said that while no great accuracy can be claimed for these constants, it seems very improbable that any other selection of complex ions would have explained the results, and that part of the support for the present picture indeed comes from the relative sizes of these constants.

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SOME FERRIC HALIDE COMPLEXES, AND TERNARY COMPLEXES WITH THIOCYANATE IONS[1]

By M. W. Lister and D. E. Rivington

ABSTRACT

A spectrophotometric study of solutions containing ferric and bromide ions; ferric, bromide, and thiocyanate ions; and ferric, chloride, and thiocyanate ions is reported. The measurements were made at constant temperature and ionic strength. The data were interpreted with the help of the results from the first paper of this series. The data show that $FeBr^{++}$ and $FeBr_2^+$ are formed, and values are obtained for the equilibrium constants for their formation from simple ions, and for the extinction coefficients of $FeBr^{++}$. When thiocyanate is also present, the data can best be interpreted by assuming that $FeSCNBr^+$ or $FeSCNCl^+$ is formed. At high chloride concentrations it seems necessary to postulate also $FeCl_2^+$ and $FeSCN.Cl_2$. Estimates are made for the equilibrium constants for the formation of all these species from simple ions. As with the corresponding sulphate complexes, there seems to be no particular reluctance to form mixed complexes such as $FeSCNCl^+$, as compared with the 'simple' complexes such as $FeCl_2^+$.

In the second paper of this series (3*b*), results were reported which showed that the color of ferric thiocyanate could only be used as a means of investigating complexes of ferric and sulphate ions, if allowance were made for the presence of species containing ferric, sulphate, and thiocyanate, such as $FeSO_4.SCN$. The present work deals with a similar investigation of solutions containing chloride or bromide ions, instead of sulphate. The intention was to make a critical examination of this method of investigating ferric complexes, and in particular to discover to what extent ternary complexes, such as $FeSCN.Cl^+$ or $FeSCN.Br^+$, were important. Rabinowitch and Stockmayer have already investigated solutions containing ferric and chloride or bromide ions, but not thiocyanate as well (6). They suggested that the discrepancy between their result for the equilibrium constant for the formation of $FeCl^{++}$, and the earlier results of Bent and French (1), who examined solutions made from ferric chloride and potassium thiocyanate, was due to the ion $FeSCN.Cl^+$. The present work attempts a more quantitative estimate of the formation of this ion, and of the analogous $FeSCN.Br^+$. It is believed that the results demonstrate the existence of these ions, and values for the equilibrium constants of their formation from simple ions have been obtained.

APPARATUS AND MATERIALS

All optical measurements were made with a Beckmann Model DU spectrophotometer, modified so that the temperature of the solutions could be controlled, as described in the first paper of this series (3*a*).

The reagents used were the same as described in this same paper, with the following additions.

Hydrochloric acid.—The concentrated reagent grade acid was diluted to give a stock solution of about 1 *M* concentration. This was standardized volumetrically against borax.

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Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario.

Sodium chloride.—The reagent grade chemical was dissolved to give a stock solution of about 5 *M* concentration. This was standardized volumetrically against silver nitrate.

Sodium bromide.—A pure grade of sodium bromide was dissolved to give a 5 *M* stock solution, which was standardized by titration against silver nitrate.

The general technique used in making up and storing solutions, in preventing fading of the ferric thiocyanate color, and in measuring the optical densities was exactly the same as described in the first paper (3*a*). The mixtures in the present work contained ferric perchlorate, perchloric acid (to suppress hydrolysis), sodium perchlorate (to adjust the ionic strength), sodium thiocyanate, and sodium chloride or bromide.

EXPERIMENTAL RESULTS

It was first necessary to check that the ions $FeCl^{++}$ or $FeBr^{++}$ did not absorb light appreciably in the region of measurement which was 4400 to 4800 Å. Solutions containing 0.001 *M* ferric ions in 0.2 *M* perchloric acid and a large excess of sodium chloride (about 0.6 *M*) show a strong absorption band with a maximum at 3270 Å; however the absorption falls rapidly at longer wave lengths, and is negligible at 4400 Å. Similar measurements with bromide substituted for chloride show only a very slight absorption in the ultraviolet besides that due to ferric ions, but there is a long 'tail' extending into the visible with a very weak subsidiary maximum at about 4100 Å. Accordingly solutions containing ferric, bromide, and thiocyanate ions were measured against a 'reagent blank' which contained the same concentrations of the various ions except for thiocyanate. Some measurements were also made on ferric bromide solutions (without thiocyanate) in which case the reagent blank was simply a ferric perchlorate solution. These measurements with ferric, bromide, and thiocyanate solutions against this reagent blank require a slight correction which will be described later.

Effect of chloride ion.—In order to investigate the effect of chloride ion on the ferric thiocyanate equilibrium, a series of solutions having 0.001 *M* total iron, 0.00125 *M* total thiocyanate, 0.200 *M* hydrogen ions, an ionic strength of 1.2, and total chloride varying from zero to 1.2 *M* were prepared. The optical densities of these solutions at 25°C. were measured from 4400 to 4800 Å. The results are given in Table I.

Effect of bromide ion.—As the $FeBr^{++}$ complex has only been examined once before (6), it was thought worth while to check the value of its equilibrium constant. Accordingly measurements were made on solutions containing ferric perchlorate, perchloric acid, sodium perchlorate, and sodium bromide only, without thiocyanate. The results are given in Table II. The value of the dissociation constant of $FeBr^{++}$, and its extinction coefficients, will be deduced in the next section.

Solutions containing ferric, thiocyanate, and bromide ions were also examined. The results are given in Table III; as was mentioned above, these results are against a reagent blank of the same composition as the test solutions except that the thiocyanate is omitted.

TABLE I

OPTICAL DENSITIES OF SOLUTIONS CONTAINING FERRIC, CHLORIDE, AND THIOCYANATE IONS (Fe^{+3}) = 0.001 M; (SCN^-) = 0.00125 M; (H^+) = 0.2 M; ionic strength = 1.2; light path = 1 cm.; temperature 25°C.

(Cl^-), M	Wave length, mμ				
	440	450	460	470	480
0	.562	.592	.599	.587	.557
0.025	.539	.568	.572	.561	.530
0.050	.511	.541	.549	.535	.503
0.075	.492	.519	.528	.512	.483
0.100	.471	.498	.502	.492	.464
0.125	.459	.481	.489	.479	.452
0.150	.447	.469	.472	.462	.439
0.200	.419	.441	.448	.438	.411
0.250	.395	.417	.422	.411	.389
0.300	.370	.390	.396	.386	.365
0.350	.352	.371	.376	.368	.346
0.400	.336	.353	.359	.350	.330
0.450	.322	.339	.344	.336	.318
0.500	.309	.325	.329	.323	.304
0.600	.284	.299	.304	.297	.280
0.700	.266	.281	.286	.279	.265
0.800	.250	.265	.269	.262	.248
1.000	.225	.239	.241	.235	.222
1.200	.205	.216	.220	.215	.204

TABLE II

OPTICAL DENSITIES OF SOLUTIONS CONTAINING FERRIC AND BROMIDE IONS (Fe^{+3}) = 0.001 M; (H^+) = 0.2 M; ionic strength = 1.2; light path 1 cm.; 25°C.

(Br^-), M	Wave length, mμ					
	380	400	420	440	450	460
0.10	.025	.028	.025	.020	.016	.013
0.20	.047	.054	.050	.037	.030	.025
0.30	.065	.075	.068	.050	.041	.034
0.40	.084	.095	.088	.065	.054	.044
0.50	.099	.113	.105	.078	.064	.051
0.60	.115	.131	.120	.091	.075	.060
0.70	.130	.148	.136	.102	.085	.066
0.80	.143	.161	.149	.110	.092	.075
0.90	.154	.174	.160	.120	.099	.080
1.00	.161	.184	.167	.125	.105	.085

TABLE III

OPTICAL DENSITIES OF SOLUTIONS CONTAINING FERRIC, BROMIDE, AND THIOCYANATE IONS (Fe^{+3}) = 0.001 M; (SCN^-) = 0.001 M; (H^+) = 0.2 M; ionic strength 1.2; temp. 25°C.; light path 1 cm.

(Br^-), M	Wave length, mμ					
	400	420	440	450	460	470
0	.234	.360	.459	.481	.488	.478
0.10	.223	.345	.440	.466	.471	.461
0.20	.210	.328	.424	.449	.457	.447
0.30	.200	.318	.409	.431	.439	.429
0.40	.193	.305	.395	.420	.428	.420
0.50	.185	.294	.381	.406	.411	.406
0.60	.176	.285	.368	.391	.401	.394
0.70	.170	.273	.355	.380	.386	.381
0.80	.156	.254	.333	.356	.366	.361
0.90	.150	.246	.324	.345	.354	.350
1.00	.141	.232	.305	.327	.334	.330

DISCUSSION OF RESULTS

1. Solutions Containing Bromide

The results in Table II will be considered first as these are most simply dealt with. The method of Rabinowitch and Stockmayer (6) can be used to interpret these results. If we use the following symbols:

a = total ferric concentration,
b = total bromide concentration,
D = optical density,
ϵ = extinction coefficient of $FeBr^{++}$,
k = equilibrium constant of $FeBr^{++} = (FeBr^{++})/(Fe^{+3})(Br^{-})$;

then Rabinowitch and Stockmayer show that

$$b/D = b/a\epsilon + 1/ka\epsilon$$

holds to a good approximation. In Table II, a is kept constant and b is varied; hence a plot of b/D against b should give a straight line from which k and ϵ can be evaluated. The results of Table II, when plotted in this way, do give reasonably straight lines; and by drawing lines by the method of least squares through the experimental points, we obtain the values of k and ϵ given in Table IV. The best average value of k is probably 0.61, as the result at 4500 Å

TABLE IV

VALUES OF k AND ϵ FOR $FeBr^{++}$

λ, mμ	ϵ	k
380	435	0.601
400	491	0.606
420	457	0.594
440	328	0.630
450	295	0.562
460	225	0.607

is somewhat divergent. This can be compared with the value of 0.5 obtained by Rabinowitch and Stockmayer. The values for the extinction coefficients make the absorption maximum close to 4050 Å, and the maximum extinction coefficient is about 495. This is lower than the value obtained by Rabinowitch and Stockmayer, but the reason for the discrepancy is not clear.

The equation given above assumes that $FeBr^{++}$ is the only complex formed. At high concentrations there is a slight but consistent deviation from the optical densities calculated on the above assumptions. This is presumably due to $FeBr_2^{+}$ being formed. If we call the equilibrium constant of this ion k_2, where $k_2 = (FeBr_2^{+})/(Fe^{+3})(Br^{-})^2$, and ϵ_2 is its extinction coefficient, and if we assume that at these high concentrations the concentration of free bromide is virtually equal to the total bromide, then

$$a(\epsilon k + \epsilon_2 k_2 b) = D(1/b + k + k_2 b).$$

This equation contains two unknowns, and so these can be evaluated from any two measurements at one wave length. Unfortunately a very small error

in the density can lead to a considerable error in the value of k_2. If we take the points at $b = 1.0$ and 0.7 as the two measurements to calculate k_2, we get the following values of k_2 from the measurements at various wave lengths:

λ	3800	4000	4200	4400	4500	4600 Å
k_2	0.17	0.18	0.24	0.43	0.07	0.19

An error of 0.001 in the density could give an error of 0.1 in k_2; so the most that can be said is that k_2 is probably close to 0.2. The values of ϵ_2 are not reliable enough to be worth reporting: they seemed to run parallel to ϵ, but to be unduly low.

Turning now to the results when both bromide and thiocyanate are present, we shall attempt to explain them on the assumption that the complex ions $FeBr^{++}$, $FeSCN^{++}$, and $FeSCN.Br^{+}$ are present, and at higher concentrations $FeBr_2^{+}$ also. A rough calculation showed that the observed densities did not agree with those calculated if only $FeBr^{++}$ and $FeSCN^{++}$ are formed, so that some other ion must be formed; we shall be justified in assuming that this is $FeSCN.Br^{+}$ if the results give a reasonably constant value for its equilibrium constant. It will readily be appreciated that this system leads to rather complicated expressions for the constants, so that some approximations have to be made. If the concentrations of the various species are indicated by the following letters: $(Fe^{+3}) = p$, $(SCN^{-}) = q$, $(FeSCN^{++}) = x$, $(Br^{-}) = r$, $(FeBr^{++}) = u$, $(FeSCN.Br^{+}) = z$, there are three equations for the equilibrium constants of the complex ions:

$$FeBr^{++} : k_a = u/pr,$$
$$FeSCN^{++} : k_1 = x/pq,$$
$$FeSCN.Br^{+} : k_m = z/pqr.$$

The total iron (a), total bromide (c), and total thiocyanate (b) in the solution lead to equations for the mass balances:

$$a = p+x+u+z,$$
$$b = q+x+z,$$
$$c = r+u+z.$$

Finally the observed density is given by

$$D' = \epsilon_1 x+\epsilon_m z+\text{correction},$$

where ϵ_1 and ϵ_m are the extinction coefficients of $FeSCN^{++}$ and $FeSCN.Br^{+}$. The correction is small owing to the fact that there is a slightly different amount of $FeBr^{++}$ in the reagent blank and in the test solution, as some of the ferric ions are used up forming $FeSCN^{++}$ in the test solution. This correction was evaluated from a rough value of k_m (and of course the other constants) in a way that will be described in a moment. There are thus seven equations and six concentrations, so that in theory the two unknown constants, ϵ_m and k_m, can be found from any two measurements.

The results in Table III are for solutions in which $a = b$, and the total bromide (c) is much larger than a or b. Therefore it seemed reasonable to put

$r = c$, as a good approximation. If D is the corrected optical density, given by

$$D = \epsilon_1 x + \epsilon_m z,$$

we can eliminate p, r, u, x, and z from the above equations to get:

[i] $$\frac{q^2(k_1+k_m c)}{1+k_a c} + q - a = 0,$$

[ii] $$q = \frac{D(1+k_a c)}{a(\epsilon_1 k_1 + \epsilon_m k_m c) - D(k_1 + k_m c)}.$$

These make use of the fact that, in these solutions, $a = b$.

Before dealing with these equations further, we must explain how D' was corrected to give D. The correction is evidently $\epsilon_a(u_0 - u)$, where ϵ_a is the extinction coefficient of $FeBr^{++}$ obtained above, and u_0 is the concentration of this ion when no thiocyanate is present. Putting $b = 0$, u_0 is readily obtained as

$$u_0 = k_a ca/(1+k_a c).$$

When thiocyanate is present, as $a = b$:

$$u = k_a cq/(1+k_a c);$$

and equation [i] can be converted into a quadratic in u:

$$u^2(k_1+k_m c) + u.k_a c - [a(k_a c)^2/(1-k_a c)] = 0.$$

Very rough calculation showed that k_m was not far from 25, and this value was used to obtain u and hence $u_0 - u$. The values of $u_0 - u$ so obtained were

c	0.1	0.2	0.4	0.6	0.8	1.0
$u_0 - u$	0.62	1.05	1.82	2.40	2.73	3.03×10^{-5}

Hence as ϵ_a is known the correction can be calculated; it is always less than 0.015, usually much less, and not particularly sensitive to changes in k_m: hence the rough value of k_m was quite adequate here.

Before we turn to the main method used to determine k_m, a word should be said about applying the initial slope method, used for the mixed sulphate–thiocyanate complexes in the second paper of this series. This method assumed that the mixed complex (in that case $FeSO_4.SCN$) absorbed light to nearly the same extent as $FeSCN^{++}$, at least in the region of the main absorption band of the latter. However $FeBr^{++}$ also absorbs somewhat in the region where $FeSCN^{++}$ absorbs, (while $FeSO_4^+$ absorbs there scarcely at all), so it cannot be assumed that $FeSCN^{++}$ and $FeSCNBr^+$ have nearly the same extinction coefficient. Actually if this assumption is made, the equilibrium constant for $FeSCNBr^+$ comes out as 30, while (to anticipate) the most reliable value is probably 21. Accordingly a more laborious, but probably more accurate, method was used to evaluate k_m.

Equations [i] and [ii] above are the starting point of the method. q could be eliminated readily enough, but the resulting equation in ϵ_m and k_m is very cumbersome. A method of successive approximations was therefore used, as follows. First let us assume that $\epsilon_1 = \epsilon_m$; in spite of the considerations given

in the last paragraph, this is unlikely to be totally wrong, in view of the formulae of the ions concerned, and at the next approximation we can discard this assumption. If $\epsilon_1 = \epsilon_m$, equations [i] and [ii] can be readily combined, and reduced to

$$k_1+k_mc = \frac{(1+k_ac)D/\epsilon_1}{(a-D/\epsilon_1)^2}.$$

Hence a rough value of k_m can be obtained from each density reading, and with this value of k_m, q can be calculated from equation [i]; then everything in equation [ii] is known except ϵ_m, which can thus be evaluated to a first approximation. With these values of ϵ_m and k_m, improved values of q can be obtained from equation [ii], and then used in equation [i] to obtain improved values of k_m. In theory these approximations could be repeated; in effect we are adjusting k_m and ϵ_m until the two equations give the same values of q for each value of c, the total bromide concentration. However the first approximation $\epsilon_1 = \epsilon_m$ is actually a fairly good approximation so the process did not need to be carried very far.

Table V gives the results of the first approximation for k_m. Ignoring the rather deviant values at 4000 Å, k_m averages as 21.2. The first approximation for ϵ_m gives the values in Table VI. These values are not very constant, but it is easily seen that a relatively small change in D will give a considerable change in ϵ_m. The absorption band of $FeSCNBr^+$ is evidently similar to that of $FeSCN^{++}$ shifted somewhat to the red. If we use these mean values of ϵ_m from Table VI, we get as our next approximation for k_m the results in Table VII. The results in Table VII are more consistent but the average is little changed.

TABLE V

FIRST APPROXIMATE VALUES FOR k_m

c	Wave length, mμ					
	400	420	440	450	460	470
0.1	31.5	27.5	25.7	26.5	24.2	31.4
0.2	13.2	15.2	22.2	22.8	27.1	29.3
0.3	12.0	20.5	21.0	18.4	21.3	21.9
0.4	15.4	17.9	20.0	21.5	23.5	25.6
0.5	14.8	17.2	18.3	20.0	19.1	23.0
0.6	12.0	17.9	17.1	17.7	20.4	21.9
0.7	14.7	14.8	15.4	17.6	17.6	19.9

TABLE VI

FIRST APPROXIMATE VALUES OF ϵ_m

c	Wave length, mμ					
	400	420	440	450	460	470
0.1	(3100)	4290	4930	5570	5360	(6340)
0.2	1540	2650	4560	4920	5760	5990
0.3	1420	3350	4350	4110	4680	4680
0.4	1720	2990	4170	4680	5110	5350
0.5	1660	2900	3880	4410	4330	4880
0.6	1410	2990	3670	3980	4540	4680
Mean	1530	3190	4260	4610	4960	5120
ϵ_m/ϵ_1	0.69	0.93	0.97	0.995	1.06	1.12

TABLE VII

VALUES OF k_m; SECOND APPROXIMATION

	Wave length, mμ					
c	400	420	440	450	460	470
0.1	(46.9)	29.4	24.9	26.1	23.1	26.7
0.2	21.3	17.2	22.8	22.7	24.9	25.1
0.3	19.5	22.3	21.7	18.7	19.9	19.2
0.4	24.3	19.7	20.7	21.5	21.9	22.2
0.5	23.2	19.1	19.1	20.2	18.3	20.1
0.6	19.3	19.8	18.0	18.0	19.2	19.2
0.7	19.7	—	16.4	17.9	16.8	17.6
Mean	21.2	21.2	20.5	20.75	20.6	21.45

The best value for k_m is 21, probably 21 ± 2. The constancy of the values in Table VII seems to be sufficient to justify the initial assumption that the ion $FeSCNBr^+$ is indeed present. It is scarcely worth correcting the ϵ_m values further; however it was found that if k_m is reduced to 21.0, ϵ_m is raised by about $\frac{3}{4}\%$. Therefore the best values of ϵ_m, rounded off to the nearest 50, are:

λ	4000	4200	4400	4500	4600	4700 Å
ϵ_m	1550	3200	4300	4650	5000	5150

At high bromide concentrations some deviation occurs, probably owing to the appearance of $FeBr_2^+$. An estimate of its effect can be made as follows. With the constants already known the densities at high bromide concentrations can be calculated; no systematic deviations from experiment were found until $[Br^-]$ is 0.7, but after this the observed densities were consistently higher. If we attribute this to $FeBr_2^+$, which removes some of the iron from possible equilibrium with $FeSCN^{++}$, the concentration of $FeBr_2^+$ can be calculated, and hence its equilibrium constant, k_2, which was defined earlier. The mean value of k_2 at various wave lengths was:

k_2	0.29	0.18	0.19	0.20	0.17
λ	4200	4400	4500	4600	4700 Å

This makes the mean value of k_2 about 0.2. This is a very rough calculation; but as the result agrees with the same constant obtained from solutions without thiocyanate, it seems plausible that this really is the cause of the deviation.

2. Solutions Containing Chloride

The first constant for the association of ferric ions with chloride ions has been measured before, e.g. Rabinowitch and Stockmayer (6), Bray and Hershey (2), Moeller (4), Olerup (5). These authors all used solutions which did not contain thiocyanate, and hence interference by ions such as $FeSCNCl^+$ could not occur. The best value for this constant, $K_1 = (FeCl^{++})/(Fe^{+3}) \cdot (Cl^-)$, under the conditions of the present experiments is probably 4.1. Both Rabinowitch and Stockmayer, and Moeller get results very close to this. Bent and French (1), using mixtures containing ferric, chloride, and thiocyanate ions, arrived at a value of 1.3; this difference is presumably due to $FeSCNCl^+$ ions. Our own results in Table I do not agree with the calculated densities, assuming that K_1 is 4.1 and no $FeSCNCl^+$ is present; hence we shall assume that $FeSCNCl^+$ really is present and attempt to evaluate its equilibrium constant.

Examination of Table I shows that as the chloride concentration is increased the densities at all wave lengths are reduced by the same fractional amount, at least to within the experimental error. Hence $FeSCNCl^+$ must have very nearly the same absorption spectrum as $FeSCN^{++}$; all the extinction coefficients of $FeSCNCl^+$ could be larger than those of $FeSCN^{++}$, but they would all have to be larger in the same ratio. It can also be seen that at any one wave length the densities fall off at first by an amount proportional to the chloride added. When the total chloride exceeds about 0.1 M, the fall slowly becomes less steep. The interpretation of the results was attempted by two methods: (i) as for the bromide solutions above, and (ii) as for the sulphate solutions, described in the second paper of this series. As a result of applying the first method, the values for ϵ_M, the extinction coefficient of $FeSCNCl^+$, which were obtained were all within 1% of those for $FeSCN^{++}$, which is within the experimental error. This is not surprising since $FeCl^{++}$ scarcely absorbs at all in this region. Since this is so, we can apply the second method, that is, the initial slope of the plot of D against total chloride as a means of determining k_M. Here k_M, the equilibrium constant for $FeSCNCl^+$, is defined as

$$k_M = (FeSCNCl^+)/(Fe^{+3}).(SCN^-).(Cl^-).$$

The evaluation of k_M is very similar to the method used for sulphate; but as here the concentrations of iron and thiocyanate are not equal, and as there is no complication from ions such as $FeHSO_4^{++}$, it is perhaps worth outlining the calculation. If we write for the concentrations:

$$(Fe^{+3}) = p, \quad (SCN^-) = q, \quad (FeSCN^{++}) = x,$$
$$(Cl^-) = r, \quad (FeCl^{++}) = u, \quad (FeSCNCl^+) = z,$$

we have from the Mass Law:

$$k_1 = x/pq, \quad K_1 = u/pr, \quad k_M = z/pqr,$$

where k_1, K_1, and k_M are the equilibrium constants for $FeSCN^{++}$, $FeCl^{++}$, and $FeSCNCl^+$ respectively. If a, b, and c are respectively the total concentrations of iron, thiocyanate, and chloride present, mass balances give:

$$a = p+x+u+z,$$
$$b = q+x+z,$$
$$c = r+u+z.$$

If ϵ_1 and ϵ_M are the extinction coefficients of $FeSCN^{++}$ and $FeSCNCl^+$, then

$$D = \epsilon_1 x+\epsilon_M z.$$

We shall assume for the reasons given above that $\epsilon_1 = \epsilon_M$. Eliminating x, u, and z:

$$a = p(1+k_1 q+K_1 r+k_M qr),$$
$$b = q(1+k_1 p+k_M pr),$$
$$c = r(1+K_1 p+k_M pq),$$
$$D = pq\ \epsilon_1(k_1+k_M r).$$

Differentiating these with respect to c, and (as we are interested in the initial slope) putting $c = r = 0$ in the result, we get

$$0 = \frac{\partial p}{\partial c}(1+k_1 q)+p\left(k_1\frac{\partial q}{\partial c}+K_1\frac{\partial r}{\partial c}+k_M q\frac{\partial r}{\partial c}\right),$$

$$0 = \frac{\partial q}{\partial c}(1+k_1 p)+q\left(k_1\frac{\partial p}{\partial c}+k_M p\frac{\partial r}{\partial c}\right),$$

$$1 = \frac{\partial r}{\partial c}(1+K_1 p+k_M pq),$$

$$\frac{\partial D}{\partial c} = \epsilon_1 k_1\left(p\frac{\partial q}{\partial c}+q\frac{\partial p}{\partial c}\right)+pq\epsilon_1 k_M\frac{\partial r}{\partial c}.$$

The initial concentrations, p_0 and q_0, are easily found as

$$a = p_0(1+k_1 q_0),$$
$$b = q_0(1+k_1 p_0).$$

D_0, the initial density, equals $\epsilon_1 k_1 p_0 q_0$. The equation in the initial slope of D reduces to

$$\frac{1}{D_0}\frac{\partial D}{\partial c} = \frac{1}{p_0}\frac{\partial p}{\partial c}+\frac{1}{q_0}\frac{\partial q}{\partial c}+\frac{k_M}{k_1}\frac{\partial r}{\partial c}.$$

Substituting for $\partial p/\partial c$, $\partial q/\partial c$, and $\partial r/\partial c$ from the equations above and simplifying, the result is finally:

$$\frac{1}{D_0}\frac{\partial D}{\partial c} = \frac{k_M/k_1-K_1}{(1+K_1 p_0+k_M p_0 q_0)(1+k_1 p_0+k_1 q_0)}.$$

The values of $(1/D_0)\,\partial D/\partial c$ at various wave lengths are

λ	4400	4500	4600	4700	4800 Å
$-(1/D_0)\partial D/\partial c$	1.62	1.59	1.62	1.62	1.67

The mean value is -1.62. With this and the values of a and b (and hence p_0 and q_0) from Table I, we get $k_M = 260$, to two significant figures. An error of about 1% in the slope would lead to about 1% error in k_M.

As the density falls off more gradually at high chloride concentrations it is evident that some other colored species must then have appeared. We can feel sure that $FeCl_2^+$ must be formed to some extent, and the new colored species is presumably $FeSCN.Cl_2$. As the position and shape of the absorption maximum does not change at high chloride concentration, it is reasonable to assume to a first approximation that the extinction coefficients of $FeSCNCl_2$ are roughly the same as those of $FeSCN^{++}$. With this assumption it is possible to evaluate the equilibrium constants of $FeCl_2^+$ and $FeSCNCl_2$. Let these constants be

$$k_2 = (FeCl_2^+)/(Fe^{+3}).(Cl^-)^2,$$
$$k_n = (FeSCNCl_2)/(Fe^{+3}).(SCN^-).(Cl^-)^2.$$

When the chloride concentration is high, it must be nearly true that $c = r$. The equations given above for the Mass Law, etc., can then be reduced to:

$$X(1+K_1 c+k_2 c^2) = (a-X)(b-X)(k_1+k_M c+k_n c^2)$$

where $X = D/\epsilon_1$. The results of Table I give virtually the same values of X at all wave lengths for a given value of c. The average values of X are as follows (using the values of ϵ_1 given in the first paper in this series):

X	1.015	0.956	0.902	0.846	0.804	0.766	0.735	0.705	0.649	0.610	0.574	0.515	0.470 $\times 10^{-4}$
c	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.60	0.70	0.80	1.0	1.2

The equation above in X contains two unknowns, k_2 and k_n. It could be written in the form:

$$Ak_2 = B + k_n$$

where

$$A = X/(a-X)(b-X),$$

$$B = (1/c^2)\{k_1 + k_M c - A(1+K_1 c)\}.$$

The values of A and B obtained from the data are:

c	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.60	0.70	0.80	1.0	1.2
A	98.4	91.6	85.5	79.3	74.8	70.7	67.4	64.3	58.6	54.6	51.1	45.3	41.0
B	484	409	370	363	333	309	284	265	240	212	193	164	143

A plot of A against B gives a reasonably straight line from $c = 0.3$ to 0.8. At lower c values the points are rather erratic, and the last two points drift away a little from the line. The fact that the plot is linear over a considerable length is some evidence that this is the correct explanation. The line makes $k_2 = 6.1$ and $k_n = 120$.

Finally it seems worth while to summarize all the constants evaluated for the complex ions considered in this work. The constants are the equilibrium constants for the formation of the complex ions from simple ions, written with the concentration of the complex ion on top. They are at the same ionic strength.

$FeSCN^{++}$	130;	$FeCl^{++}$	4.1;	$FeBr^{++}$	0.61;	$FeSO_4^+$	170;	
$Fe(SCN)_2^+$	1950;	$FeCl_2^+$	6.1;	$FeBr_2^+$	0.2;	$Fe(SO_4)_2^-$	17000;	
$FeSCNCl^+$	265;	$FeSCNBr^+$	21;	$FeSCN.SO_4$	12000;	$FeHSO_4^{++}$	6;	
$FeSCNCl_2$	120;			$FeSCN(SO_4)_2^{--}$	490000;	$FeHSO_4.SO_4$	380	

By dividing these constants, the constants for the attachment of further simple ions to the various complex ions can be obtained. As is to be expected, further ions are held less firmly than the first one attached; and generally speaking the numbers so obtained seem fairly consistent, or, at least, provide no obvious contradictions. The chief point to note is that the mixed ions, such as $FeSCNCl^+$, are formed as readily (or very nearly so) as those derived from only two simple ions, such as $FeBr_2^+$. This is perhaps slightly unexpected, but it is difficult to think of any reason why this should not be so.

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THEORY OF THE TRANSIENT PHASE IN KINETICS, WITH SPECIAL REFERENCE TO ENZYME SYSTEMS[1]

By Keith J. Laidler[2]

ABSTRACT

The steady-state hypothesis is discussed for enzyme systems, and the conditions under which the steady-state equations will be valid over the main course of the reaction are obtained. It is shown that this is so if the substrate is in great excess, and also under several other circumstances. Equations are derived for the kinetic behavior during the transient phase of the reaction. Two-substrate systems, and the special case of catalase, are considered.

In the theoretical formulation of the rates of complex reactions in terms of the concentrations of reactants and other substances (e.g. inhibitors) it is customary to make use of the steady-state hypothesis. This hypothesis, according to which the rates of change of the concentrations of reaction intermediates can be neglected, is easily justified for systems in which the intermediates are present in much lower concentrations than the reactants. In enzyme systems, however, this condition is not necessarily satisfied, since in many instances the concentration of intermediate—the enzyme–substrate complex—is close to the total concentration of the enzyme.

The object of this paper is to examine the steady-state hypothesis as applied to simple enzyme systems, and to see under what conditions it may be expected to be valid. Consideration is also devoted to the question of the kinetics during the transient phase of the reaction—the period during which the steady-state concentration is being established. A single-substrate system is treated first, after which a two-substrate system is briefly considered.

Some of the kinetic equations obtained in this paper have previously been derived, particularly by Chance (3, 4, 5) and by Beers (1, 2), but the methods here employed are more general and permit application to a wider variety of cases.

THE SINGLE-SUBSTRATE SYSTEM

The single-substrate system, uncomplicated by the effects of activators and inhibitors, may be represented by the Michaelis-Menten scheme:

$$\underset{e_0-y}{\mathrm{E}} + \underset{s_0-y-p}{\mathrm{S}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{y}{\mathrm{ES}} \xrightarrow{k_2} \underset{e_0-y}{\mathrm{E}} + \underset{p}{\mathrm{P}}.$$

Here E represents enzyme, S the substrate, P the product or products of reaction, and ES the binary complex between enzyme and substrate. If e_0 is the *total* concentration of enzyme (including that bound to substrate), s_0 the *initial total* concentration of substrate, p the amount of product at time t, and y the amount of complex at time t, the various concentrations at time t are as represented above. The rate equations are therefore

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[1] $$dy/dt = k_1(e_0-y)(s_0-y-p)-\bar{k}y$$

and

[2] $$dp/dt = k_2y$$

where $\bar{k}$ is equal to $k_{-1}+k_2$.

Steady-state Treatment

The steady-state treatment involves setting dy/dt equal to zero in equation [1] and solving for y. The result is

[3] $$y=\left[\bar{k}+k_1(e_0+s_0-p)\pm[\bar{k}+k_1(e_0+s_0-p)]\left(1-\frac{4k_1^2e_0(s_0-p)}{[\bar{k}+k_1(e_0+s_0-p)]^2}\right)^{\frac{1}{2}}\right]\Big/ 2k_1.$$

In this solution the negative sign must be taken, as otherwise it becomes possible for y to be greater than e_0. In the event that

[4] $$[\bar{k}+k_1(e_0+s_0-p)]^2 \gg 4\,k_1^2e_0(s_0-p)$$

we can expand the square root and accept only the first term; the solution then reduces to

[5] $$y = \frac{k_1e_0(s_0-p)}{\bar{k}+k_1(e_0+s_0-p)}.$$

We may now enquire under what circumstances the condition [4] is satisfied. The inequality [4] transforms into

[6] $$\bar{k}^2+k_1^2[e_0^2+(s_0-p)^2]+2\bar{k}k_1(e_0+s_0-p) \gg 2k_1^2e_0(s_0-p)$$

or into

[7] $$\frac{\bar{k}^2}{2k_1^2e_0(s_0-p)}+\frac{e_0}{2(s_0-p)}+\frac{s_0-p}{2e_0}+\frac{\bar{k}(e_0+s_0-p)}{k_1e_0(s_0-p)} \gg 1.$$

This relationship can be true in various ways. Since p cannot be greater than s_0, it is evident that [7] is true if

[8] $$s_0 \gg e_0$$

or if

[9] $$e_0 \gg s_0.$$

It is also true if

[10] $$\bar{k} \gg k_1e_0$$

or if

[11] $$\bar{k} \gg k_1s_0.$$

The inequality [8] of course corresponds to a situation that usually exists in enzyme systems. However even if s_0 and e_0 are of comparable magnitudes equation [5] is valid provided that either [10] or [11] is true. If $s_0 \gg e_0$ equation [5] can be written as

[12] $$y = \frac{k_1e_0(s_0-p)}{\bar{k}+k_1(s_0-p)}$$

and the steady-state rate is then

$$[13] \qquad \frac{dp}{dt} = \frac{k_1 k_2 e_0 (s_0 - p)}{k + k_1 (s_0 - p)}.$$

The Maximum Concentration of Complex

It is unfortunately not possible to obtain an exact explicit solution of the differential equations [1] and [2]. It is possible, however, to arrive at some conclusions about the circumstances under which the steady-state assumption will be reasonably accurate.

For this purpose it is convenient to note that the relationships derived in the last section are satisfactory in giving the *maximum* concentration of complex, y_m, in terms of the concentration of product, p_m, present at the maximum. Thus if in equations [3], [5], [12], and [13] we replace y by y_m and p by p_m we obtain equations relating y_m and p_m which are independent of the steady-state assumption; the relationship

$$[14] \qquad y_m = \frac{k_1 e_0 (s_0 - p_m)}{k + k_1 (e_0 + s_0 - p_m)},$$

for example, is true provided that one of the four conditions [8], [9], [10], and [11] is satisfied. It is clear, therefore, that the steady-state equations are *exact* at the maximum. As the reaction proceeds the steady-state assumption predicts only a gradual diminution in y as p increases, and the exact theory predicts such a change during the later stages of the reaction. The steady-state assumption will, however, lead to error in the early stages of reaction, when the concentration of complex is building up, but if this occurs during the *very* early stages of the reaction the error will not be considerable over the main course of reaction.

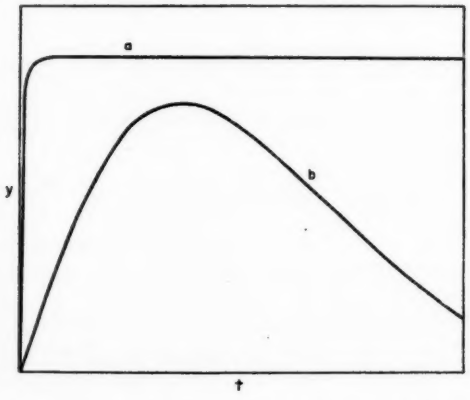

FIG. 1. Schematic curves showing concentration of enzyme–substrate complex vs. time. In curve *a* the steady state is established rapidly; in *b* the concentration of complex varies significantly throughout the course of reaction.

In view of this it is desirable to obtain an estimate of the time taken for the maximum concentration to be attained, in order to see whether it represents an appreciable fraction of the entire reaction. One may envisage two extreme cases, as represented in curves *a* and *b* of Fig. 1. In curve *a* the maximum is reached in the very early stages of the reaction, and in this case the steady-state treatment is satisfactory for the most part. In curve *b* on the other hand, the maximum is a fairly sharp one and the steady-state assumption is therefore unjustified.

The Transient Phase

A kinetic expression will now be obtained for the behavior during the transient phase. The equation will of necessity be an approximate one, but will be sufficiently correct to allow dependable conclusions to be drawn with regard to the circumstances under which the transient phase will constitute a small fraction of the half-life of the reaction.

In place of equation [1] we start with the approximate equation

$$[15] \qquad dy/dt = k_1(e_0-y)(\bar{s}-y)-\bar{k}y.$$

In this equation s_0-p has been replaced by $\bar{s}$, which is treated as constant; $\bar{s}$ may be regarded as the average concentration of the substrate during the transient phase. The circumstances under which this will be a close approximation are discussed later. Equation [15] integrates to

$$[16] \qquad t = \frac{1}{\sqrt{Q}} \ln \frac{2ay+b-\sqrt{Q}}{2ay+b+\sqrt{Q}} + \text{constant}$$

where

$$[17] \qquad Q = b^2-4ac,$$

$$[18] \qquad a = k_1,$$

$$[19] \qquad b = -(k_1e_0+\bar{k}+k_1\bar{s}),$$

$$[20] \qquad c = k_1e_0\bar{s}.$$

The boundary condition $t = 0$, $y = 0$ gives rise to

$$[21] \qquad t = \frac{1}{\sqrt{Q}} \ln \frac{by+2c+y\sqrt{Q}}{by+2c-y\sqrt{Q}}.$$

At the maximum

$$[22] \qquad ay_m^2+by_m+c = 0,$$

whence

$$[23] \qquad y_m = (-b-\sqrt{Q})/2a.$$

From equations [17] and [23] it can be shown that

$$[24] \qquad \sqrt{Q} = -ay_m+(c/y_m),$$

$$[25] \qquad b = -ay_m-(c/y_m).$$

The rate equation [21] therefore reduces as follows:

$$t = \frac{1}{\sqrt{Q}} \ln \frac{c - a y_m y}{c - cy/y_m} \quad [26]$$

$$= \frac{1}{\sqrt{Q}} \ln \frac{y_m - a y y_m^2/c}{y_m - y} \quad [27]$$

$$= \frac{1}{[(k_1 e_0 + \bar{k} + k\bar{s})^2 - 4k_1^2 e_0 \bar{s}]^{\frac{1}{2}}} \ln \frac{y_m(1 + y y_m/e_0\bar{s})}{y_m - y} \quad [28]$$

$$= \frac{y_m}{k_1 e_0 \bar{s} - k_1 y_m^2} \ln \frac{y_m(1 + y y_m/e_0\bar{s})}{y_m - y}. \quad [29]$$

It is now possible to arrive at an estimate of the amount of product, p_m, that has been produced during the transient phase. The time that it takes for y to reach $0.99y_m$ is given by (from equations [27] and [28])

$$\tau = \frac{1}{\sqrt{Q}} \ln 100\left(1 + \frac{y y_m}{e_0 \bar{s}}\right). \quad [30]$$

Since y and y_m are necessarily less than the smaller of e_0 and $\bar{s}$ it follows that

$$\tau < (1/\sqrt{Q}) \ln 200 \quad [31]$$

$$< 5.3/\sqrt{Q}. \quad [32]$$

Since y is always less than y_m during the induction period, an *upper limit* to the rate of formation of product is therefore given by

$$dp/dt < k_2 y_m. \quad [33]$$

This integrates to

$$p < k_2 y_m t \quad [34]$$

since $p = 0$ when $t = 0$. An upper limit to the amount of product produced until $y = 0.99y_m$ (and therefore essentially an upper limit to the amount of product produced in the transient phase) is therefore given by

$$p < 5.3\, k_2 y_m/\sqrt{Q}. \quad [35]$$

Using equations [17] and [23] this becomes

$$p < \frac{5.3k_2}{2a}\left(-\frac{b}{\sqrt{Q}} - 1\right) \quad [36]$$

$$< \frac{5.3k_2}{2k_1}\left(-\frac{b}{\sqrt{Q}} - 1\right), \quad [37]$$

$$\frac{p}{s_0} < \frac{5.3k_2}{2k_1 s_0}\left[\frac{1}{\sqrt{(1 - 4ac/b^2)}} - 1\right]. \quad [38]$$

From this it follows that p/s_0 is small, i.e. that not much product has been produced during the transient phase, if

$$4ac \ll b^2. \quad [39]$$

The circumstances under which this is so were considered earlier, and are expressed by the inequalities [8]–[11] above. It may further be noted that b^2 is necessarily greater than $4ac$ (cf. [6] above), so that the term in the square brackets in [38] can never become very large; p/s_0 will therefore also be small if

[40] $$k_1 s_0 \gg k_2.$$

The conclusion that has been reached is therefore that the transient phase will be a relatively short one provided that one or more of the inequalities [8], [9], [10], [11], and [40] are satisfied. The situation is then as represented in curve *a* of Fig. 1. In any of these circumstances the kinetic equations derived in the present section for the transient phase will be obeyed with high accuracy since the approximation involved in equation [15] is justified *a posteriori*.

A Special Case: The Substrate Is Present in Great Excess

In view of the fact that in most enzyme systems the concentration of enzyme is very much less than that of the substrate it is now convenient to write down those relationships that are particularly applicable to this case. For this purpose the starred quantities, y^*, y_m^*, p^* will be used when the relationships between them only apply in the case that $s_0 \gg e_0$.

The value of y_m^* is given by (cf. equation [12])

[41] $$y_m^* = \frac{k_1 e_0 s_0}{\bar{k}+k_1 s_0} .$$

During the induction period the rate equations are

[42] $$dy^*/dt = k_1 s_0 (e_0 - y^*) - \bar{k} y^*,$$

[43] $$dp^*/dt = k_2 y^*.$$

Since in equation [29] $k_1 y_m^{*2} \ll k_1 e_0 \bar{s}$ and $y y_m \ll e_0 \bar{s}$ (since y and y_m must be less than e_0, which is much less than $\bar{s}$), and $\bar{s}$ can be replaced by s_0, the kinetic law during the transient phase is

[44] $$t = \frac{y_m^*}{k_1 e_0 s_0} \ln \frac{y_m^*}{y_m^* - y^*} .$$

This may be written as

[45] $$y^* = y_m^* [1 - \exp(-k_1 e_0 s_0 t / y_m^*)]$$

and using equation [41] this becomes

[46] $$y^* = \frac{k_1 e_0 s_0}{\bar{k}+k_1 s_0} [1 - \exp\{-(\bar{k}+k_1 s_0)t\}].$$

The variation of the concentration of product during the transient phase is obtained by using equation [43]:

[47] $$\frac{dp^*}{dt} = \frac{k_1 k_2 e_0 s_0}{\bar{k}+k_1 s_0} [1 - \exp\{-(\bar{k}+k_1 s_0)t\}].$$

With the boundary condition $t = 0$, $p = 0$ this integrates to

$$[48] \qquad p^* = \frac{k_1k_2e_0s_0}{\bar{k}+k_1s_0}t+\frac{k_1k_2e_0s_0}{(\bar{k}+k_1s_0)^2}[\exp\{-(\bar{k}+k_1s_0)t\}-1].$$

When t is sufficiently large, i.e. at the maximum, the kinetics are seen to be first order. At lower times more complex behavior is observed. If t is sufficiently small that

$$[49] \qquad (\bar{k}+k_1s_0)t \ll 1$$

it is permissible to expand the exponential and accept only the first two terms; the result is

$$[50] \qquad p^* = \tfrac{1}{2}k_1k_2e_0s_0t^2.$$

This particular relationship has previously been given by Roughton (8).

Equations for the rate of disappearance of substrate during the transient phase can readily be obtained by making use of the fact that

$$[51] \qquad -ds/dt = (dp/dt)+(dy/dt).$$

Another result of interest is the time that it takes for the steady state to be established. Equation [32] gives the time that it takes for y to reach 0.99 y_m, and for the special case of $s_0 \gg e_0$ we obtain

$$[52] \qquad \tau \simeq 5.3/(\bar{k}+k_1s_0).$$

It is of interest that this time is independent of the concentration of the enzyme. An experimental determination of the length of the induction period should therefore allow an estimate to be made of the magnitude of $\bar{k}+k_1s_0$, i.e. of $k_{-1}+k_2+k_1s_0$, and since k_2 and $k_1/(k_{-1}+k_2)$ can be determined by conventional kinetic methods the constants can be separated. Work along these lines has been carried out by Gutfreund (6). A more accurate, but experimentally more difficult, procedure for separating constants is to follow the rate of increase of y^* during the induction period; $\bar{k}+k_1s_0$ can then be determined using equation [46].

After y has attained the value of y_m the steady state is established, and the remaining course of the reaction is in accordance with equation [13]. This equation integrates to

$$[53] \qquad k_1k_2e_0 = \frac{1}{t}\ln\frac{s_0}{s_0-p}+\frac{k_1p}{\bar{k}t},$$

which is equivalent to the well-known Henri equation (7). The behavior is zero order as long as $k_1(s_0-p) \gg \bar{k}$ and first order when $\bar{k} \gg k_1(s_0-p)$.

The conclusion that has been reached in the present treatment is that as long as $s_0 \gg e_0$ the kinetic behavior can be closely represented by two sets of

equations, one referring to the transient phase and the other to the steady-state period. The situation is represented schematically in Fig. 2. Here the curve ABC corresponds to equation [46] for the transient phase, the value of y approaching y_m^* asymptotically. The curve DBF, corresponding to equation [12], is that predicted by the steady-state theory. The treatment proposed in the present paper involves regarding ABF as representing the true course of the reaction. Strictly speaking it gives a discontinuity at B, but since y_m is very close to y_m^* the error is evidently very slight.

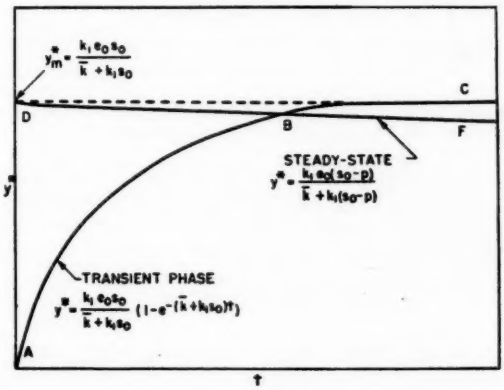

FIG. 2. Schematic curves showing complex concentration vs. time, for the case in which the substrate concentration is in great excess. The true course of the reaction is closely represented by ABF.

The above treatment at once leads to a useful graphical procedure for arriving at the variation of y with t in terms of known rate constants and initial conditions. The curve ABC can readily be plotted, and from equation [48] the amount of product at various times can be calculated. Curve DBF can then be constructed, and ABF will then represent the course of the reaction to a high approximation.

It may be noted that curves and equations of the same *form* as in Fig. 2 and the present section apply also if any of the conditions [9], [10], [11], and [40] apply. The constants appearing in the equations are, however, different for these cases, but may easily be written down by applying the methods of the present section.

The General Case

When none of the conditions [8], [9], [10], [11], and [40] applies it is no longer the case that only a small fraction of the substrate has been transformed into product by the time y has reached its maximum value. The situation is now more as represented in Fig. 3. The true curve, AGF, now deviates considerably from the transient-phase and steady-state curves in the region of

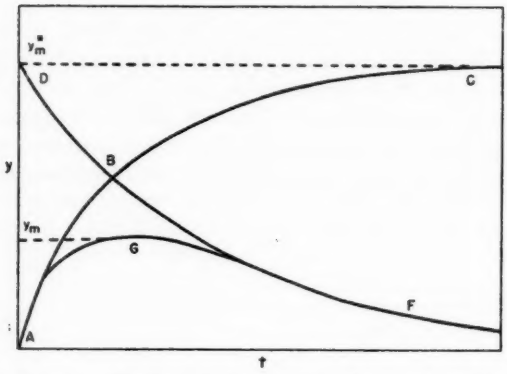

FIG. 3. Schematic curves showing complex concentration vs. time, for the case in which none of the inequalities [8], [9], [10], [11], and [40] is applicable.

the maximum, but corresponds at the beginning and end of the reaction. In the very early stages of the reaction equation [28] is valid and since y and p are small it may be written as

$$[54] \qquad t = \frac{1}{[(k_1e_0+\bar{k}+k_1s_0)^2-4k_1^2e_0s_0]^{\frac{1}{2}}} \ln \frac{y_m^*}{y_m^*-y}$$

or

$$[55] \qquad y = y_m^*(1-\exp\{-[(k_1e_0+\bar{k}+k_1s_0)^2-4k_1^2e_0s_0]^{\frac{1}{2}}t\})$$

where y_m^* is defined by equation [41]. This equation is of the same form as equation [46], and enables the function in the square brackets to be determined from the experimental results. A further separation of constants would, however, appear to be very difficult for this general case without the use of a differential analyzer.

TWO-SUBSTRATE SYSTEMS

A somewhat similar type of analysis can be applied to systems in which there are two substrates. The simplest kinetic scheme corresponding to this situation is the following:

$$\underset{e_0-y}{E} + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{s_0-y-p}{} \underset{y}{ES},$$

$$\underset{y}{ES} + \underset{r_0-p}{R} \xrightarrow{k_2} \underset{e_0-y}{E} + \underset{p}{P}.$$

Here R, the second substrate, does not form a ternary complex ESR but reacts with ES to give products in a single step; the case of the ternary complex is considerably more difficult to work out. Examples of the above scheme are to be found in the peroxidatic reactions of peroxidase and catalase; in these reactions S is hydrogen peroxide and R an acceptor molecule.

The rate equations for this system are

[56] $$dy/dt = k_1(e_0-y)(s_0-y-p)-k_2(r_0-p)y-k_{-1}y,$$

and

[57] $$dp/dt = k_2y(r_0-p).$$

Steady-state Treatment

The general steady-state equation for this system is equation [3] with $\bar{k}$ replaced by $k_2(r_0-p)+k_{-1}$. In the event that any one of the conditions [8], [9], [10], and [11] holds the steady-state equation reduces to

[58] $$y = \frac{k_1e_0(s_0-p)}{k_{-1}+k_1(e_0+s_0-p)+k_2(r_0-p)}.$$

According to the steady-state treatment the concentration of y therefore starts at an initial value of

[59] $$y_0 = \frac{k_1e_0s_0}{k_{-1}+k_1(e_0+s_0)+k_2r_0}$$

when $t = 0$ and gradually diminishes as product accumulates.

The situation is again that steady-state conditions may, under certain circumstances, be expected to exist during the main course of the reaction. When this is the case a simple rate equation will apply to the early stages of the reaction, as will now be considered.

The Transient Phase

The procedure will again be to employ an approximate rate equation for the first part of the reaction, and to justify it *a posteriori* under certain circumstances.

The equation that will be used is

[60] $$dy/dt = k_1(e_0-y)(\bar{s}-y)-(k_2\bar{r}+k_{-1})y$$

where $\bar{s}$ and $\bar{r}$ are the average amounts of S and R during the transient phase. This equation is seen to be of the same form as equation [15], and the remainder of the argument is therefore exactly as previously except that $\bar{k}$ is now given the significance of $k_2\bar{r}+k_{-1}$. The general kinetic equation for the transient phase is now (cf. equation [28])

[61] $$t = \frac{1}{[(k_1e_0+k_1\bar{s}+k_2\bar{r}+k_{-1})^2-4k_1^2e_0s_0]^{\frac{1}{2}}} \ln \frac{y_m(1+yy_m/e_0\bar{s})}{y_m-y}$$

where y_m is as given previously (except that $\bar{k}$ is now $k_2\bar{r}+k_{-1}$). The conclusion is again that during the induction period only a very small amount of product is produced provided that one or more of the conditions [8], [9], [10], [11], and [40] are satisfied.

In the event that the two substrates are greatly in excess of the enzyme the rate equation during the induction period is (cf. equation [46])

[62] $$y^* = \frac{k_1e_0s_0}{k_{-1}+k_1s_0+k_2r_0}[1-\exp\{-(k_{-1}+k_1s_0+k_2r_0)t\}].$$

The course of the formation of product can then be readily obtained by proceeding as previously (equations [47]–[50]). Chance (3) has previously treated this type of situation using numerical methods.

THE CATALATIC REACTION

A slight variant of the above treatment is provided by the catalatic reaction —the decomposition of hydrogen peroxide catalyzed by the enzyme catalase. The reaction scheme is now (4)

$$\underset{e_0-y}{E} + \underset{s_0-y-p}{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{y}{X},$$

$$\underset{y}{X} + \underset{s_0-y-p}{S} \xrightarrow{k_2} \underset{e_0-y}{E} + \underset{p}{P}.$$

The rate equations for this system are

[63] $$dy/dt = k_1(e_0-y)(s_0-y-p)-k_2y(s_0-y-p)-k_{-1}y,$$

[64] $$dp/dt = k_2y(s_0-y-p).$$

In the event that one of the conditions equivalent to [8], [9], [10], or [11] holds the steady-state equation reduces to

[65] $$y = \frac{k_1e_0(s_0-p)}{k_{-1}+(k_{-1}+k_2)(s_0-p)}.$$

Chance (4, 5) has shown that k_{-1} is exceedingly small so that almost invariably it is true that

[66] $$y = k_1e_0/(k_1+k_2).$$

The Transient Phase

In order to obtain an approximate expression for the transient phase the procedure will be, as above, to write equation [63] as

[67] $$dy/dt = k_1(e_0-y)(\bar{s}-y)-k_2y(\bar{s}-y)$$

where $\bar{s}$ is the average amount of free substrate during the transient phase. The solution of this is equations [16] and [21] above, but a, b, and c now have a slightly different significance, namely

[68] $$a = k_1+k_2,$$

[69] $$b = -(k_1e_0+k_1\bar{s}+k_2\bar{s})+k_1e_0\bar{s},$$

[70] $$c = k_1e_0\bar{s}.$$

The remainder of the treatment is then as given previously. It again follows that the transient phase will be short compared with the over-all duration of the reaction if, among other possibilities, $s_0 \gg e_0$.

For this case the final result for the kinetic equation during the transient phases is again found to be equation [44]. Such a result has previously been obtained, using another procedure, by Chance *et al.* (5) and by Beers and Sizer (2).

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NOTES

A NEW TYPE OF ULTRAFILTER

By J. L. Gardon[1] and S. G. Mason

INTRODUCTION

Most ultrafilters (2, 3, 6) have perforated or porous plates as membrane supports, use membranes of small area, and to get appreciable rates of filtration are operated at relatively high pressures. The solution, being under pressure, cannot readily be stirred and, becoming increasingly concentrated on the membrane surface, has a tendency to block the membrane pores (1). Such ultrafilters are not suitable for handling large quantities of materials.

A simple and inexpensive method of supporting membranes which was developed by the authors for an osmometer cell and which inhibits ballooning in membranes as large as 200 sq. cm. in area is described elsewhere (4). In the present note we describe an ultrafiltration unit consisting of a number of slightly modified osmometer cells which has been used for the fractionation of ligninsulphonates (5).

Since the membrane area is large, the unit may be used at relatively low pressure differentials using water aspirators. It is fully automatic, simple to construct, leakproof, and allows thorough stirring of the liquid.

ULTRAFILTER CELLS

Each ultrafilter cell consists mainly of two lucite disks and two rubber rings (Fig. 1). The membrane is clamped between the rubber rings, reinforced with fine-mesh stainless steel screens, and supported on both sides by two layers of tightly packed glass beads. To avoid leakage at the edges of the screens, rings of dental dam rubber are placed between them and the membrane and between them and the rubber rings, forming the body of the cell. When the unit is compressed, the dental dam penetrates into the meshes of the screen reinforcers and seals the cell effectively. The necessary parts for a typical cell are described in Table I.

A simple leakproof connection between the glass parts and the lucite can be made without cement. For this purpose tygon tubing with 1.5 mm. wall thickness and 10 mm. O.D. is placed into 8 mm. bore holes in the lucite. To introduce the tubing into the hole the end of it is cut off conically along the length and pulled into the hole by the tongue thus formed, and the tongue is then cut away.

Each lucite disk is equipped with the connectors before assembling the cell. To prevent glass beads from dropping into the tygon tubing, a small piece of stainless steel screen is placed in the tubing.

[1]*Holder of the Anglo-Paper Research Fellowship and of a Studentship from the National Research Council of Canada. Present address: Industrial Cellulose Research Ltd., Hawkesbury, Ontario.*

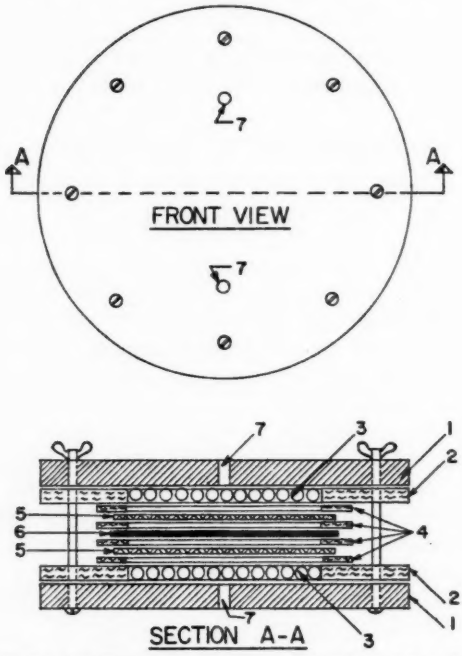

FIG. 1. Details of the ultrafilter cell: (1) lucite disks, (2) rubber rings, (3) glass beads, (4) dental dam gaskets, (5) stainless steel screens, (6) membrane, (7) inlet hole.

TABLE I

CELL COMPONENTS

1. 2 lucite disks
   - Diameter 25 cm.
   - Thickness 1 cm.
   - 8 holes with centers on a 23 cm. diameter circle concentric with the disks to accommodate the screws
   - 2 holes with centers on the same diameter 17.5 cm. apart—bore diameter 8 mm.
2. 8 rubber rings
   - Inside diameter 18 cm.
   - Outside diameter 25 cm.
   - Thickness 0.3 to 0.32 cm.
   - 8 holes drilled in identical positions as in the lucite for holding the screws
3. 4 dental dam gaskets
   - Inside diameter 18 cm.
   - Outside diameter 22 cm.
   - Thickness about 0.03 cm.
4. 2 stainless steel screens
   - Diameter 20 cm.
   - 125 mesh
5. 1 membrane
   - Diameter 21 cm.
6. Glass beads
   - Diameter 0.28 to 0.3 cm.
7. 8 screws, 8 wing nuts, 16 washers

THE CIRCULATING PUMP

Stirring in the cells is provided by circulating the solution from a reservoir. The pumping unit, shown in detail in Fig. 2, consists of a membrane pump (A) governed by an oscillating mercury pump (B).

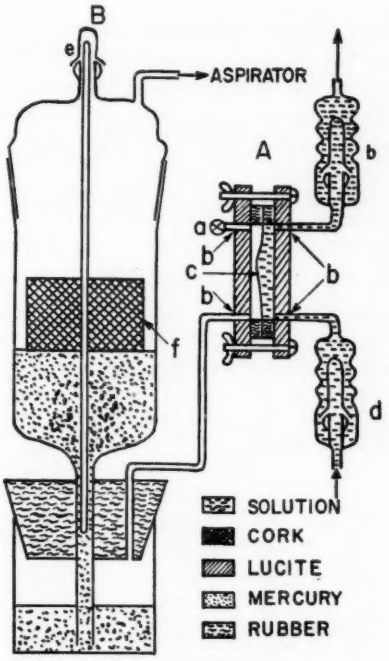

FIG. 2. Details of the circulating pump: (A) membrane pump, (a) stopcock, (b) inlet holes, (c) dental dam membrane, (d) valves, (B) oscillating mercury pump, (e) valve, (f) cork floater.

The construction of the membrane pump is similar to that of the ultrafilter cells. It is made up of two lucite disks of 12 cm. diameter, two rubber rings 5 mm. thick with 10 cm. outside diameter and 8 cm. inside diameter, and an elastic dental dam membrane disk (c) of 10 cm. diameter clamped between the rubber rings. To accommodate the screws, both lucite disks have four holes with centers on an 11 cm. diameter circle concentric with the disks and two 8 mm. bore holes (b), with centers 3.75 cm. from the center of the disk and on the same diameter, in which tygon tubing is placed in the same manner as described above. The membrane is made of two dental dam rubber sheets stuck together with silicone grease. This method of preparation gives the membrane a life of at least several months of continuous operation.

The two check valves (d) are made of spherical glass joints of 12 mm. diameter.

The oscillating mercury pump functions as follows. By applying vacuum in the upper chamber, mercury is sucked into it. The rising mercury raises the cork float (f) and thus opens valve (e). Consequently air enters into the upper chamber, the mercury level drops, valve (e) is closed, and a new pumping cycle starts. The lower chamber of this pump is connected with the membrane pump, and the membrane in the latter forcibly has to follow the movements of the mercury. In practice the oscillating mercury pump is put into

operation first, independently of the membrane pump, and stopcock (a) is closed afterwards. The difference in level between the mercury in the lower and upper chambers should alternate between 10 and 14 cm. The diameter in both the upper and lower chambers of the oscillating mercury pump is 7 cm. and the length of the pump is 25 cm. The valve (e) is made of a 12 mm. diameter spherical glass joint.

The capacity of this pumping unit is around 200 cc. per minute.

OPERATION

The assembled ultrafilter is shown in Fig. 3. The solution chambers of the different cells are connected in series and the solution is circulated from reservoir (C) to provide adequate stirring.

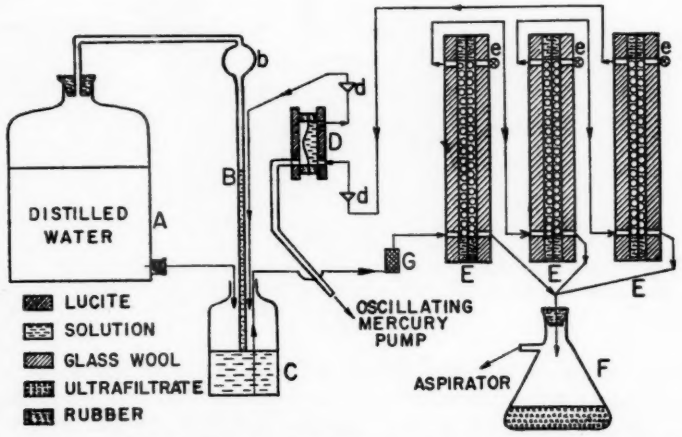

FIG. 3. The assembled ultrafiltration unit: (A) water reservoir, (B) constant head device, (b) solution trap, (C) solution reservoir, (D) membrane pump, (d) valves, (E) ultrafilter cells, (e) stopcocks, (F) suction flask, reservoir for ultrafiltrate, (G) glass wool filter.

The volume of the liquor is kept constant by the constant head device (B). If the level in reservoir (C) drops sufficiently to expose the end of the tube (B) to the air, air will enter into the distilled water container (A) and consequently water will flow into reservoir (C). The rising level in the reservoir closes tube (B) and thus stops the water flow. Trap (b) is needed to prevent the solution from entering the distilled water container.

The ultrafiltrate is fed into suction flask (F) by gravity.

Keeping the residual liquor at constant volume allows good control of the process. If V_0 is the volume of the residual liquor, K the volume of ultrafiltrate produced in unit time, $c(t)$ the concentration of the component to be removed, and $\gamma\, c(t)$ its concentration in the ultrafiltrate passing through the membrane at time t (γ is the partition coefficient between the solutions inside and outside

the membrane, $0 < \gamma < 1$), the following relation may be derived for the variation of $c(t)$ with time:

$$-V_0\,dc = \gamma\,K\,c(t)\,dt,$$

which on integration yields

$$c(t) = c(0).e^{-\gamma K t/V_0}.$$

By determining K, V_0, c_0, and several values of $c(t)$ at different time intervals, γ can be evaluated and the time necessary for the removal of any arbitrary portion of the component in question can be calculated.

The rate of ultrafiltration is readily varied by changing the number of cells in the unit.

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THE REMOVAL OF FLUORIDE ION FROM AQUEOUS SOLUTION BY MAGNESIUM OXIDE

BY JOHN C. BARTLET AND ROSS A. CHAPMAN[1]

Venkateswarlu and Narayana Rao (1, 2, 3) reported that magnesium oxide takes up fluoride ion from hot solution, basic to phenolphthalein. Light magnesium oxide was reported to remove up to 20 μgm. of fluoride per mgm. of magnesium oxide. This procedure was used to reduce the fluoride content of water (1, 2) and as an aid in the determination of fluoride in tea (3).

In this laboratory, it was found that the removal of fluoride could be increased manyfold if the original solution was acidic. Table I shows the effect

TABLE I

THE EFFECT OF pH ON THE TAKING UP OF FLUORIDE FROM AQUEOUS SOLUTION BY MAGNESIUM OXIDE

pH		% Removal of fluoride
Before addition of MgO	After addition of MgO	
12.5	12.4	10
11.7	11.6	50
8.6	10.5	88
5.6	10.3	94
1.7	8.9	100

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of pH on the taking up of 1 mgm. of fluoride ion in 100 ml. of hot solution by 100 mgm. of magnesium oxide. The pH of the solutions was adjusted with sodium hydroxide or perchloric acid. The solution of pH 1.7 contained sufficient perchloric acid to dissolve approximately 40 mgm. of the original 100 mgm. of magnesium oxide added. From a hot solution at pH 1.7 containing 20 mgm. of fluoride as sodium fluoride in 100 ml., 100 mgm. of magnesium oxide took up 18.8 mgm. or 94% of the fluoride. This represents a removal of 188 μgm. of fluoride per mgm. of original magnesium oxide or 312 μgm. of fluoride per mgm. of magnesium oxide remaining in the solid phase.

The solid residue prepared under several different conditions with an excess of fluoride present was found to be of variable composition ranging from approximately the composition $2Mg(OH)_2 \cdot MgF_2$ to $4Mg(OH)_2 \cdot 3MgF_2$. These products were slightly soluble in hot water. Examination by the X-ray diffraction powder method showed that the products consisted of a mixture of $Mg(OH)_2$ and MgF_2 with no lines attributable to a new compound.

We wish to thank Dr. W. H. Barnes of the National Research Council of Canada who carried out the X-ray diffraction studies.

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A SYNTHESIS OF L-ISOGLUTAMINE FROM γ-BENZYL GLUTAMATE

BY M. KRAML AND L. P. BOUTHILLIER

In the course of our studies on histidine metabolism (5) it became necessary to prepare some L-isoglutamine. Bergmann and Zervas (1) obtained the desired product by cleaving N-carbobenzyloxy-L-glutamic anhydride with anhydrous ammonia followed by catalytic hydrogenation to remove the carbobenzyloxy (CBZ) group. Recently, Chambers and Carpenter (3) achieved a similar synthesis by blocking the amino acid with the *p*-nitrobenzyloxycarbonyl group. Paper chromatography (3) has revealed that these methods, while favoring L-isoglutamine formation, invariably yield small amounts of L-glutamic acid and L-glutamine as contaminants. Swan and duVigneaud (6) have published the only unambiguous synthesis of L-isoglutamine. It consists in the preparation of N-*p*-toluenesulphonyl-L-glutamic acid, the conversion to N-tos-5-oxo-2-pyrrolidine-carboxyl chloride, the amidation to N-tos-5-oxo-2-pyrrolidine-carboxamide, the conversion to N-tos-L-isoglutamine, and the subsequent hydrogenation with sodium in liquid ammonia to give L-isoglutamine. We have found it more convenient to prepare L-isoglutamine by the amidation of N-CBZ-γ-benzyl-L-glutamate by the mixed anhydride procedure

of Boissonnas (2), followed by catalytic hydrogenolysis of the resulting amide. The method is evidently unambiguous and L-isoglutamine is the only product formed. This was verified by paper chromatography in four solvent systems.

EXPERIMENTAL[1]

N-CBZ-γ-benzyl-L-glutamate

γ-Benzyl-L-glutamate, m.p. 168–169°, was condensed with carbobenzyloxy chloride in the presence of sodium bicarbonate as suggested by Hanby *et al.* (4). The N-CBZ-γ-benzyl-L-glutamate was crystallized from hot carbon tetrachloride, m.p. 77–78°. Calculated for $C_{20}H_{21}NO_6$, N: 3.77. Found N: 3.75.

N-CBZ-γ-benzyl-L-isoglutamine

N-CBZ-γ-benzyl-L-glutamate (32.8 mM.) was dissolved in 150 ml. of dioxane (redistilled from sodium) containing 7.80 ml. of tri-*n*-butylamine. The solution was cooled to 10° and 3.16 ml. of ethyl chlorocarbonate was added. The solution was shaken for 10 min. at 10° after which anhydrous ammonia was bubbled through for 15–30 min. The solution was evaporated to a white powder on a water bath at a temperature not exceeding 50°. The residue was suspended in 50 ml. of carbon tetrachloride and 50 ml. of water was added. The mixture was agitated vigorously and the N-CBZ-γ-benzyl-L-isoglutamine was filtered off and washed with cold water, carbon tetrachloride, and ether to remove any trace of starting material. The compound was recrystallized from hot ethanol, m.p. 126–127°, yield 80%. N-CBZ-γ-benzyl-L-isoglutamine has not been previously described in the chemical literature. Calculated for $C_{20}H_{22}N_2O_6$, C: 64.86; H: 5.96; N: 7.56. Found C: 65.16; H: 6.19; N: 7.52.

L-*Isoglutamine*

N-CBZ-γ-benzyl-L-isoglutamine (5 gm.) was dissolved in 100 ml. of 50% ethanol and hydrogenated for 12 hr. at room temperature and atmospheric pressure in the presence of palladium catalyst (5% Pd on calcium carbonate). The catalyst was filtered off and a trace of calcium ions was removed with oxalic acid. The solution was evaporated to dryness, the residue dissolved in a small amount of hot water, and the solution treated with charcoal. Alcohol was added to the cloud point and L-isoglutamine was recovered from the chilled solution as fine white crystals, m.p. 186–188°, yield 65%. Calculated for $C_5H_{10}N_2O_3$, N: 19.16. Found N: 18.94. Chambers and Carpenter (3) reported a melting point of 175–176°, whereas Swan and duVigneaud (6) found 186°. Chambers and Carpenter have reported $[\alpha]_D^{24}$ +19.4 to 20.5 (*c* 3, water) and Swan and duVigneaud, $[\alpha]_D^{21}$ +20.5° (*c* 6.1, water). We have measured $[\alpha]_D^{24}$ +20.0° (*c* 2.3, water). Paper chromatograms of our product revealed only one bluish-purple spot in each of the four solvent systems employed. The R_f in *n*-butanol – glacial acetic acid – water (15:3:7) was 0.28 and there was no

[1] *Analyses by the Geller Microanalytical Laboratories, Hackensack, New Jersey. All melting points were taken by the capillary method and are uncorrected.*

trace of L-glutamine (R_f 0.18); the R_f in phenol–water (4:1) was 0.56 and L-glutamic acid (R_f 0.30) was absent.

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Contents

	Page
Carbohydrate Thioethers. 1-Deoxy-1-thioethyl-D-fructose—*Samuel B. Baker*	1459
The Terminal Amino Acids of Wheat Gliadin—*L. K. Ramachandran and W. B. McConnell*	1463
The Preparation of Guanidine from Urea, Ammonium Chloride, Aluminum Sulphate, and Ammonia Under Pressure—*Jean L. Boivin*	1467
Synthesis of γ-Hydroxyglutamic Acid (Diastereomeric Mixture)—*Léo Benoiton and L. P. Bouthillier*	1473
Physicochemical Studies of Ligninsulphonates. I. Preparation and Properties of Fractionated Samples— *J. L. Gardon and S. G. Mason*	1477
Physicochemical Studies of Ligninsulphonates. II. Behavior as Polyelectrolytes—*J. L. Gardon and S. G. Mason*	1491
Studies on Alumina Catalysts. IV. The Production of Diethylaniline by Condensation of Ethanol and Ethylaniline—*L. A. Munro and R. A. Washington*	1502
Conductances of Aqueous Lithium Nitrate Solutions at 25.0°C. and 110.0°C.—*A. N. Campbell, G. H. Debus, and E. M. Kartzmark*	1508
Steroids and Related Products. III. The Synthesis of 17β-Methyl-17-isodesoxycorticosterone—*Ch. R. Engel and G. Just*	1515
Carbohydrates of Sunflower Heads—*C. T. Bishop*	1521
The Vapor-phase Photolysis of Acetic Acid—*P. Ausloos and E. W. R. Steacie*	1530
Reactions of Arylsulphonic Esters. III. On the Hydrolysis of Methyl *p*-Methylbenzenesulphonate—*R. E. Robertson*	1536
On the Temperature Dependence of the Reaction Velocity—*J. B. Hyne and R. E. Robertson*	1544
Some Correlations of the Molecular Structure of Organic Phosphorus Compounds with Their Infrared Spectra—*R. B. Harvey and J. E. Mayhood*	1552
The Analysis of Fluorocarbons: Use of Infrared Spectrophotometry for the Analysis of Small Samples—*P. B. Ayscough*	1566
Some Measurements on the Iron (111) - Thiocyanate System in Aqueous Solution—*M. W. Lister and D. E. Rivington*	1572
Ferric Sulphate Complexes, and Ternary Complexes with Thiocyanate Ions—*M. W. Lister and D. E. Rivington*	1591
Some Ferric Halide Complexes, and Ternary Complexes with Thiocyanate Ions—*M. W. Lister and D. E. Rivington*	1603
Theory of the Transient Phase in Kinetics, with Special Reference to Enzyme Systems—*Keith J. Laidler*	1614
A New Type of Ultrafilter—*J. L. Gardon and S. G. Mason*	1625
The Removal of Fluoride Ion from Aqueous Solution by Magnesium Oxide—*John C. Bartlet and Ross A. Chapman*	1629
A Synthesis of L-Isoglutamine from γ-Benzyl Glutamate—*M. Kraml and L. P. Bouthillier*	1630

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